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ACUTE INTERSTITIAL NEPHRITIS.

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PLATES XXXVII AND XXXVIII.

DEFINITION.—An acute inflammation of the kidney characterized by cellular and fluid exudation in the interstitial tissue, accompanied by, but not dependent on, degeneration of the epithelium; the exudation is not purulent in character, and the lesions may be both diffuse and focal.

This condition was first described by Biermer (1). He found the kidneys of a child four and a half years old, who died of scarlet fever, greatly swollen, the surface pale, and on microscopical examination showing intense infiltration of the connective tissue with lymphoid cells. He considers the formation of lymphoid tissue here analogous to that found in typhoid fever. The cellular infiltration is more marked in the pyramids than in the cortex. E. Wagner (2) describes a similar case, in which, however, the enlargement of the kidneys was not so marked. The consistency of the tissue was lax, the capsule easily removed, the cortex grayish-yellow, with punctate hæmorrhages. There were accumulations of lymphoid cells in the interstitial tissue most marked in the immediate vicinity of the glomeruli. In a subsequent article (3) he describes the condition under the name of acute lymphomatous nephritis, and considers it one of the four forms of acute Bright's disease. He finds this

form of nephritis most often in scarlet fever, and sometimes in typhoid. The kidneys are greatly enlarged, brittle, the capsule is easily stripped off, the surface is covered with grayish-white elevations which correspond to streaks of cellular infiltration in the cortex. There are accumulations of round cells varying in size in the interstitial tissue. Some of these cells are smaller than leucocytes, others larger. The most acute cases of scarlatinal nephritis are of the lymphomatous form. In three typical cases which Wagner describes there was pharyngeal diphtheria in addition to scarlatina.

Klebs (4) gives a good description of acute interstitial nephritis. The kidneys are enlarged, of lax consistency, the capsule is easily separated, the surface smooth and paler than normal. On section grayish-white nodules, corresponding to fatty degenerated convoluted tubules, are embedded in a rather transparent, gelatinous, grayish background. On microscopic examination the tissue between the tubules is dilated and filled with lymphoid cells. The cells come from the vessels. Letzerich (5) describes a kidney of this character in diphtheria, and regards the cells in the interstitial tissue as fungi. Coats (6), in a case of scarlet fever in an adult, finds the kidneys enlarged and similar in appearance to the leukæmic kidney. The enlargement and pallor of the kidneys are due to infiltration of the cortex with round cells. These are packed between the tubules separating them, but have not destroyed the epithelium. The microscopical appearance closely resembles leukæmia. He afterwards describes the cells in the interstitial tissue as due partly to proliferation of the interstitial cells, partly to emigration of leucocytes. The condition is common around the glomeruli in subacute nephritis.

Kelsch (7) regards acute interstitial nephritis as the typical lesion of the kidney in scarlet fever. He describes the cells as being of an embryonic character, closely packed together and often massed around the glomeruli. Klein (8, 9) found seven cases of well-marked interstitial nephritis in twenty-three cases of scarlet fever which he studied histologically. The interstitial cells are lymphoid in character; they vary in size, the nucleus is round, and two nuclei are often found in a single cell. The infiltration seems to take place first around the large vascular trunks at the base of the pyramids, and from here extends into the cortex. These cells compress the tubules, the epithelium desquamates, and the lymphoid cells enter into the tubules. In these cases albumin is often absent, and there are no symptoms pointing to implication of the kidneys. He thinks that cases which die after the ninth or tenth day of the disease will, as a general rule, show more or less well-marked interstitial changes in the kidneys.

Phisalix (10) takes the ground that acute nephritis accompanying various infectious diseases, such as scarlet fever, is not a parenchymatous, but an interstitial process. In such cases but little change may be found in the parenchyma, while the interstitial tissue is dilated and filled with cells. Weigert (11) does not recognize acute interstitial nephritis as one of the forms of acute Bright's disease. In the acute hæmorrhagic form, he describes, in addition to the hæmorrhages and epithelial degeneration, foci of brightly staining round cells in the interstitial tissue. He does not describe the character of the cells further, and considers the foci to be of an inflammatory character and secondary to the degeneration of the epithelium of the tubules and the destruction of the glomeruli. Waller (12) describes interstitial foci as common in post-scarlatinal nephritis. The cells are due to emigration, fibrous tissue may be formed from them, and a contracted kidney may be the final result of the process. Leyden (13) says that acute nephritis in scarlet fever and diphtheria is of a parenchymatous variety, but foci of small-cell infiltration are frequently found between the tubules and around the glomeruli. Bamberger (14) speaks of interstitial foci as common in the primary form of acute nephritis and in nephritis secondary to other diseases. Dunin (15) regards small-cell infiltration as one of the most characteristic changes in scarlatinal nephritis. The infiltration may affect the entire kidney or be limited to certain areas. These areas are most common close beneath the capsule or around the glomeruli. The epithelial changes may be slight, and there is no relation between epithelial degeneration and the interstitial foci. Fischl (16) finds in scarlet fever dilatation of the adventitia of the small arteries with round-cell infiltration extending from this into the tissue. In the cases of glomerulo-nephritis there is infiltration of the interstitial tissue most marked around those glomeruli which were most affected.

Friedländer (17), in his description of scarlatinal nephritis, describes interstitial septic nephritis as one of the three varieties of scarlatinal nephritis. The kidneys in this are large, lax in consistency, and the markings are obscured; the cortex is of a grayish-red opaque color, with foci of hæmorrhage. The epithelium is but little altered, and the interstitial tissue contains numbers of small round cells. This form of nephritis he finds comparatively rare, occurring only twelve times in 229 cases, and is most apt to be seen in those cases of scarlet fever which are accompanied by diphtheritic septic processes. Cornil and Brault (18) find that acute interstitial nephritis is confined to the acute febrile diseases; they have met with it in scarlet fever and other processes. Nauwerck (19,20) describes in the kidneys, in both scarlet fever and diph-

theria, foci of small-cell infiltration in the labyrinth and about the small and medium-sized veins. The intertubular capillaries may be occluded by cells derived from proliferation of the endothelium. The cells in the interstitial tissue come from emigration, but may be added to by proliferation of the fixed cells. He finds no relation between these interstitial foci and epithelial degeneration.

Langhans (21) has never found a case similar to Wagner's lymphomatous kidney in scarlet fever, but has seen a slight degree of cellular infiltration in the walls of the small veins and around the glomeruli. Crooke (22) finds distention of the capillaries in the interstitial tissue by lymphoid cells and cells derived from proliferation of the endothelium of the vessels. In many cases foci of interstitial infiltration are found both in the labryinth and close beneath the capsule. Litten (23) says that, apart from fatty degeneration and hæmorrhage, the most common lesion found in the kidney in scarlet fever is the inflammatory round-cell infiltration of the interstitial tissue. Foci of this, consisting of closely packed leucocytes, are scattered through the entire kidney, varying in extent with the intensity of the process.

Oertel (24) in his work on diphtheria describes foci of round-celled infiltration both beneath the capsule and in all parts of the tissue. Cellular infiltration is also seen in the walls of the veins and arteries. There is no connection between the accumulations of cells and desquamation of the epithelium.

Sörenson (25) gives the clinical history and the pathological anatomy of a number of cases of scarlatinal nephritis, and among them of three cases in which the interstitial lesions were well marked. In some of these cases there is a whitish opaque rim around the bases of the pyramids, with streaks extending from this into the cortex. V. Kahlden (26) gives a good description of interstitial nephritis in scarlet fever. He finds the cells in the interstitial tissue of unequal size, some smaller, some larger than leucocytes. In a case of diphtheria he finds numbers of cells in the vessels of the tissue between the cortex and the pyramids. These cells are round, in many cases twice as large as a leucocyte; the protoplasm is homogeneous and transparent. The nuclei are round or sometimes indented, and two nuclei are sometimes seen in a single cell. These cells are more frequently found in the arteries than in the veins. Baginsky and Stamm (27) find in the kidneys of scarlet fever patients who die in the first week of the disease more or less extensive interstitial infiltration with round cells. These cells are either collected into masses simulating lymph nodules or are diffused over the entire cortex.

Bernhard and Felsenthal (28) find in diphtheria, along with degenera-

tion in the epithelium, which they consider the principal change, foci of interstitial infiltration beneath the capsule around the glomeruli and in the upper portion of the pyramids. The veins are dilated and contain numbers of leucocytes. Aufrecht (29) gives the clinical history and pathological anatomy of three cases of scarlatinal nephritis which differ from the ordinary form in their early appearance and intensity. In all three cases there are masses of round cells, chiefly about the glomeruli. The changes in the parenchyma he considers independent of the interstitial lesions. Burmeister (30) describes interstitial foci in the kidneys of rabbits which were produced by the injection of chromate of ammonium into the circulation. There is no especial change in the epithelium of the tubules in the interstitial foci. He found similar interstitial foci around the glomeruli in a case of glomerulo-nephritis in a tuberculous subject. Morse (44) produced similar foci of cellular infiltration of the interstitial tissue of the kidney by injecting filtered cultures of staphylococcus aureus into the circulation of rabbits. Turner (31) makes two varieties of nephritis in scarlet fever, the glomerular and the interstitial hæmorrhagic. In the glomerular type interstitial changes are often seen. Rosenstein (32) says the large interstitial kidney, described by Friedländer, is rare in scarlet fever, although even in cases of short duration, with albuminuria from the beginning, there is evidence of interstitial proliferation with focal accumulations of cells around the vessels.

Summing up these descriptions, I find that acute interstitial lesions of the kidneys have been considered as common in scarlet fever, and are regarded by some authors as constituting the most frequent pathological alteration of the kidneys in this disease. This has also been described in diphtheria and in other infectious diseases. The cellular infiltration of the interstitial tissue may be general throughout the kidney, with more intense foci in the cortex. In these cases the kidney is greatly enlarged, in some cases twice the normal size; it is of a lax consistence, and on section is grayish and opaque. In other cases the infiltration appears as circumscribed foci either immediately beneath the capsule or around the glomeruli or at the bases of the pyramids. The cells in the interstitial tissue are almost universally described as lymphocytes, in several cases their large size and the presence of two nuclei in a single cell are mentioned. Cell accumulations have also been described around the large vessels and infiltrating their walls. Von Kahlden describes in one case accumulations of large cells

in the vessels in the pyramids. The cells have been generally considered as coming from the vessels by emigration. Almost all observers state that there does not seem to be any relation between foci of interstitial cellular infiltration and epithelial degeneration. There is general agreement that in scarlet fever the interstitial form of nephritis is most often found when the nephritis develops early in the disease, and is most common in the so-called septic cases. Many of the cases in which the most typical interstitial lesions were found are described as having also diphtheritic pharyngitis. No evidence has been given by cultures that in these cases there was mixed infection with diphtheria.

The text-books on pathology do not devote much space to this condition. Most of the authors do not recognize acute interstitial nephritis as a special type of the disease. Others speak of foci of interstitial cellular infiltration as occurring frequently in acute and chronic nephritis. In many cases the cells in the interstitial tissue are regarded as pus-cells and the condition is confounded with acute suppurative nephritis. Ziegler (33) has a very good description of the minor degrees of the process. In speaking of inflammatory œdema of the kidney he says, " the changes in the vascular stroma may take the form of circumscribed cellular infiltrations of the interlobular and circum-glomerular tissue, a condition of acute disseminated interstitial nephritis. The cellular infiltration makes its appearance in the neighborhood of the stellate and interlobular veins, and is usually so intense in these parts that in stained preparations the infiltrated patches are easily discernible under a low power of the microscope. The cellular aggregations are most numerous in the outer zone of the cortex and in the boundary zone between the cortex and medulla, the middle part of the cortex being seldom much affected." He says nothing of the character or origin of the cells, and thinks the condition may run a subacute course, gradually passing into the parenchymatous type.

The best description of the gross and microscopic condition in such kidneys is given by Orth (34). He says:

" It is rare that œdema is the most essential inflammatory change in the interstitial tissue. I have often seen kidneys in which the tubules and glomeruli were normal and in which the only change consisted in a

small-celled infiltration of the interstitial tissue. Such cases have been described as acute interstitial nephritis, as productive nephritis, as granulating nephritis, and as lymphomatous nephritis. These kidneys are more or less enlarged and softer; the surface is mottled, of a grayish-red or grayish-white color, the markings cannot be distinguished. Such kidneys can be distinguished from those of acute parenchymatous nephritis by the absence of the markings and the cloudiness of the cortex. Microscopically there is an irregular distribution of cells between the urinary tubules. These cells have a round vesicular nucleus, they are not similar to emigrated leucocytes, and I regarded them as due to growth of the connective-tissue cells, although their frequent relation to the small veins would rather speak in favor of emigration."

I have previously (35) reported eight cases of this lesion in the kidney; three of these were from scarlet fever; two from diphtheria; one followed a criminal abortion, and two were of unknown origin. In all the cellular infiltration of the interstitial tissue was the most marked, and in some cases the only change. Since then I have had the opportunity of examining microscopically the kidneys from a great many cases of diphtheria, scarlet fever, and other acute infectious diseases, and in the routine microscopic examination have found cases which showed every degree of alteration and most of which would have been overlooked without microscopic examination. The result of the further study of this important lesion in the kidney has thrown some light on the subject, and certainly caused me materially to change my views as to the origin of the cells in the interstitial tissue. In my previous communication I regarded the interstitial cells as derived from proliferation of the fixed cells of the tissue, particularly from the endothelium of the blood-vessels.

MACROSCOPIC CHANGES.

In the most marked cases the kidneys are greatly enlarged; in one case of diphtheria and measles in a child aged two years the combined weight of the kidneys was 480 grammes. In another child aged eight years, with scarlet fever, the kidneys weighed 400 grammes; in two other cases of diphtheria, one in a child of five, the other in a child of two and a half years, the kidneys weighed 250 and 225

grammes. This extreme size, which may be more than three times the normal, as in the first case mentioned, is rare. In most cases the kidneys were but slightly if at all enlarged. The appearance varies. In the most marked cases the kidney presents an appearance which can be mistaken for nothing else unless it be the leukæmic kidney. The capsule is distended, thin and easily removed, often separating spontaneously on section of the kidney. The fœtal markings are less distinct and often obliterated. The surface is pale, of a peculiar grayish-opaque color, somewhat resembling the amyloid kidney, mottled, with irregular, more hyperæmic areas; the stellate veins of the surface are injected, often showing small punctate ecchymoses around them. In some cases the surface is irregular, due to the projection of small irregular nodules which are more opaque than the surrounding tissue.

On section of such kidneys the normal markings of the cortex are obliterated, and the contrast between pyramids and cortex is less distinct. The great increase in size is principally in the cortex, which may be three or more times thicker than normal. The glomeruli are not injected, and cannot be distinguished. The general color of the cut surface is grayish and opaque, with scattered areas of injection, and a few small scattered ecchymoses. Corresponding to the elevated areas on the surface, there are often areas more opaque than the general cut surface, which extend in lines from the pyramids through the cortex. At the bases of the pyramids the same opacity of the tissue is seen. The tissue of the kidney is soft, lax, and easily broken. It is very moist, and an opaque milky fluid may be pressed out, and often flows from the cut surface. On scraping the tissue with a knife a milky fluid with small granular masses may be removed. In the less marked cases there is usually some degree of mottling, the cut surface is moist, and there are fine opaque lines or points. In many cases the changes are most evident at the bases of the pyramids and marked by a swelling and greater opacity of the tissue here. A very marked degree of change, as shown microscopically, may be present without producing macroscopic differences in the appearance of the kidney.

MICROSCOPIC CHANGES.

Sections of the fresh kidneys, to determine the presence of fatty degeneration, showed this change to be generally present, though in slight degree. Fat was found in the epithelium of the tubules, and principally in the smaller collecting tubules. In a few cases foci of more intense fatty degeneration were found in the convoluted tubules. Occasionally a slight amount of fat was found in the interstitial tissue, but not in the cells infiltrating this.

For microscopic examination thin sections of the tissue were hardened in Zenker's fluid, in corrosive sublimate, in alcohol, and in Flemming's solution. In general the best results are obtained after hardening in Zenker's fluid, and this was used in all of the routine examinations. The other solutions were only used in special cases and where the lesions were evident on macroscopic examination. Various stains were used, the most satisfactory results being obtained by staining deeply with eosin, followed by Unna's alkaline methylene-blue solution.

In the most marked cases examination with low power shows the tubules to be widely separated and an intense cellular infiltration of the interstitial tissue. This is marked in all parts, but is more intense in certain areas. These are most often situated beneath the capsule (Plate XXXVII, Fig. 1) and at the bases of the pyramids, and from these places the infiltration sometimes extends downward or upward. In the middle of the cortex there are also smaller areas of intense infiltration which seem not to be connected either with the areas beneath the capsule or with those at the base of the pyramids. In these areas the interstitial cells are closely packed together, with no intervals between them. Where the infiltration is less intense, the cells lie singly in the tissue. Around all the glomeruli, but outside of the capsules, there seems to be more marked infiltration than elsewhere, save in the areas mentioned. In the pyramids the infiltration is often as intense as in the cortex, extending down to the pelvis.

With high power (Plate XXXVII, Fig. 2) the cells in the interstitial tissue present a striking appearance. Most, and in some cases all of them, are of the same character. They vary somewhat in size

and in shape. They are generally much larger than the polymorphonuclear leucocytes, and may be from two to four times the diameter of a red blood-corpuscle. Their shape varies; the single cells not in contact with one another may be round; but where they are packed together the shape is influenced by the mutual pressure. The nuclei present some variation in size, they are never vesicular, the periphery stains deeply, and there are intensely stained points of variable size in the interior. The stained periphery often projects at certain points into the interior of the nucleus, and in most of the nuclei an intranuclear network connecting the brightly stained points within the nucleus and the points on the nuclear rim can be made out. The general body of the nucleus stains deeply. In most cases there is but a single nucleus in a cell. In others two or even three may be present. The nucleus occupies a very characteristic position. It is eccentric, and when the cells are elongated it lies usually at one of the ends. This eccentric position of the nucleus is sometimes so marked that it apparently projects from the protoplasm.

The amount of protoplasm around the nucleus varies. Most of the cells have a large amount, while in others the nuclei are found with but little surrounding them. The protoplasm is dense and often finely granular. In some of the cells the granulation is much more evident than in others. Even in the cells lying singly in the tissues the contour is not always sharp and round. In many cases both the cells here and in the masses have an irregular outline due to the protrusion of elongated and often knob-like masses of protoplasm. These present every resemblance in shape to leucocytes fixed while in emigration, and there is little doubt that these irregularities of form are due to amœboid movements. There is no appearance of a membrane around any of these cells. Their most characteristic feature is the staining of the protoplasm. With most of the nuclear stains used singly the protoplasm stains faintly, but more intensely than that of the epithelial cells. With strongly alkaline methylene-blue the protoplasm stains deeply, and the blue color is preserved after counter-staining with eosin. A faint bluish tint, giving with the eosin a lilac tint, is often seen in the protoplasm of the epithelial cells, but in the inter-

stitial cells the color is a deep blue. The cells can be instantly distinguished in a section even with a low power by their characteristic color. Nuclear figures are very numerous. As many as three or four are often seen in the field of an oil immersion. The cells in which the nuclear figures are found are large and have an abundant and distinctly granular protoplasm, the granules staining a deep blue.

These cells are found in the interstitial tissue. In all of the sections examined I have never found one of them within a tubule. In the interstitial tissue they lie loosely in the spaces of the tissue, and a few are constantly found in the blood-vessels. They are not phagocytic; neither leucocytes, red blood-corpuscles, nor detritus of other cells are found inclosed in them.

In the very marked cases, in which the kidneys are soft and enlarged, other cells are constantly found in varying numbers in the interstitial tissues. The most numerous are cells corresponding to the lymphoid cells (Plate XXXVII, Fig. 2); the nuclei of these stain very much as do the nuclei of the large cells, and every gradation can be seen between the naked nuclei of the lymphoid cells and the large cells. Polynuclear leucocytes are usually present in varying numbers, depending on the degree of degeneration in the epithelium. In a few cases, especially where the lesions are very marked and the tissue is disintegrated, I have also found cells of the epithelioid type in the interstitial tissue. These cells are, as a rule, larger, the protoplasm is finely granular, staining with eosin, the nucleus is less intensely stained than are the nuclei of the interstitial cells, and clearly vesicular. They can be easily distinguished from the blue-staining cells and there is no transition between them. These epithelioid cells, which are probably derived from proliferation of the tissue cells, are eminently phagocytic. I have found red blood-corpuscles, leucocytes, and in some cases the blue-stained cells inclosed within them. Nuclear figures are occasionally seen in the epithelioid cells, but never to the extent observed in the blue cells.

The epithelium shows various degrees of degeneration, and in places the tubules seemed to be actually destroyed.

The tubules are separated by wide areas of interstitial cellular infil-

tration, and often no tubules can be found in comparatively large areas. The slightest change found in the epithelium is a marked degree of cloudy swelling. In places the cells contain globular masses of hyaline, and the lumen of the tubule may be filled with the same material. In other cases the cells are swollen, vacuolated, and the greater part of the protoplasm may be lost, leaving only a small granular mass about the pale and rather shrunken nucleus. In places the tubules may contain desquamated epithelium or granular masses derived from it. The number of leucocytes present seems to depend upon the degree of destruction of the epithelium; they may be seen in the interstitial tissue, in the epithelial cells, and in the lumina of the tubules. The glomeruli usually show no changes, although cases of typical glomerulo-nephritis may be met with in which acute interstitial nephritis is present as a complication. Granular coagulated material may be present in the capsular space, but even this is often absent.

In the less marked cases (Plate XXXVII, Fig. 1) the general diffuse interstitial infiltration is absent. A few scattered blue-staining cells may be found, but the lesions are eminently focal. In some cases the foci are so numerous that on holding a section to the light they appear as scattered blue specks or lines (Plate XXXVIII, Fig. 3). Most of these areas, when observed with the naked eye or a very low power, have their longitudinal axes in the direction of that of the pyramids. In these foci the cells in the interstitial tissue are in great numbers, but not so numerous as in the foci in the most marked cases. These foci are principally found in three places in the tissue. As a rule, they are most numerous and largest at the bases of the pyramids, and from this point they may extend upward in the pyramidal extensions. Occasionally they are in large masses around the interlobular vessels. Next in frequency the foci are found beneath the capsule around the large branches of the stellate veins, which are often greatly dilated. Smaller foci may be found at various places throughout the cortex, but in the cortex they are most frequent around the glomeruli. The number of foci around the glomeruli varies greatly in different cases. In some there are cell masses around practically every glomerulus, in

others only scattered glomeruli are affected. I have never found a case in which the interstitial areas were confined to the tissue around the glomeruli, but there were numerous cases in which the infiltration was confined to the bases of the pyramids or to the subcapsular cortex.

These focal areas seem to stand in no relation to focal degeneration of the epithelium. In all cases degeneration of the epithelium is present. Where the infiltration is most intense there is apt to be extensive degeneration and even necrosis of the epithelium of the tubules, but this is probably secondary, and due to compression of the tubules and vessels. In these larger areas there are usually numbers of pus cells which may be found within the tubules (Plate XXXVII, Fig. 2). In the smaller areas no evidence of greater degeneration of the epithelium can be found than elsewhere in the kidney, and a high degree of degeneration may be found without any interstitial changes.

These changes in the interstitial tissue are always accompanied by changes in the vessels (Plate XXXVIII, Figs. 4, 5, 6, 7). The interstitial cells are found in the vessels as well as in the tissue, and they may be so crowded in these and in the surrounding tissue that it is difficult to distinguish the vessels. Most of the cells in the vessels have the same character and the same peculiarity of staining as the cells outside. Nuclear figures (Plate XXXVIII, Figs. 5 and 7) are found in these cells, but not in such numbers as in the cells in the interstitial tissue. There are few polynuclear leucocytes among them. The numbers of cells in the vessels are not always proportionate to the numbers in the interstitial tissue. Vessels blocked with cells may show but slight interstitial change, and occasionally interstitial foci are found where but few cells can be made out in the vessels.

In making a systematic study of the kidneys in a large number of cases of acute nephritis special attention was given to the presence of these cells in the vessels. The vessels in which the greatest number were found are always the branches of small veins in the upper part of the pyramid. In a large number of cases such cell accumulations were found in the vessels without any change in the interstitial tissue and without any more than the ordinary degeneration of the epithelium. In some cases all of the veins in this part of

the kidney were so blocked up with cells that on holding a stained specimen to the light there was a decided blue color of the upper part of the pyramid. Such cell collections were occasionally seen in the stellate veins of the cortex and elsewhere, but in many cases they were confined to the veins of the pyramids. Most of these cells in the veins were of the same character as those in the interstitial tissue. The main difference between the cells in the vessels and those in the tissue was that the vessel cells were round and regular in shape. These cell accumulations in the vessels without interstitial lesions were found in 16 out of 103 cases of pure diphtheria; in 1 case out of 19 in pure scarlet fever; in 3 out of 23 in diphtheria and scarlet fever; in 2 cases of diphtheria and measles; in 1 case of pyelo-nephritis, and in 1 case of acute endocarditis; in 24 cases in all.

There can be but one source for the cells in the interstitial tissue; they must come from the blood-vessels. In the first place, there are no cells in the tissue outside of the blood-vessels which could give origin to them. Practically the only cells which we have in the interstitial tissue of the kidney outside of the small collections of lymphoid cells, sometimes seen around the larger veins, are the cells of the blood and lymphatic vessels. It is probable that there are cells belonging to the connective tissue, but it is difficult to demonstrate their presence. These cells in the most advanced cases, in which there is actual destruction of the tissue, do proliferate and mingle with the other cells in the interstitial tissue. The resulting cells are the epithelioid cells, and can be distinguished by the nucleus, the staining of the protoplasm and their marked phagocytic properties, from the interstitial cells. Cells similar to the interstitial cells are found in the blood-vessels in the interstitial cases and in cases in which there are no interstitial lesions. It is impossible to see the irregular shapes and protrusions of the protoplasm of many of the cells in the interstitial tissue without being convinced of their amœboid properties. In a few cases similar changes of form were seen in the cells within the vessels, and in several instances such cells were found in the act of emigration, part of the cell being within and part without the vessel (Plate XXXVIII, Fig. 10). The cells are principally confined

to the vessels in the places I have mentioned. A few may be found in the larger veins, and they are occasionally seen in the vessels of the glomerulus.

The part which these cells play in pathology has received but little attention. Similar collections are most frequently described as small-celled infiltration, and no attention has been directed to their character and source. Unna was the first one who separated out from the cell collections in the interstitial tissue certain cells marked by a peculiar stain of the protoplasm, to which he gave the name plasma cells. Since then they have received considerable attention from the dermatologists and but little from pathologists. I consider the cells I have described in the interstitial tissue identical with Unna's plasma cells. Unna (36) describes in lupus cells, whose protoplasm stained deeply with alkaline methylene-blue, and which do not decolorize as the other cells. He considers them identical with the cells described by Waldeyer as plasma cells, and gives them the same name. As these cells appear in the tissue the connective-tissue cells disappear, and he supposes that they are derived from the connective-tissue cells. In his first article on the subject Unna supposes that these cells were peculiar to the tuberculous process. He subsequently (37) describes the cells more fully, modifying his first description in several particulars. In this article he considers them as greatly hypertrophied connective-tissue cells. The blue staining is due to the presence of fine granules imbedded in the substance of the cell. He finds that they play an important part in the pathology of skin diseases, and that certain new formations in the skin, to which he gives the name plasmomata, may be composed of them. The tumor cells in the round-cell sarcoma are derived exclusively from these cells, and they form the chief part of the cellular infiltration around other tumors. They are exclusively a pathological product, and are never found in embryonic or in normal tissues.

Jadassohn (38), while confirming the presence in the tissues of the cells described by Unna, denies that they are to be differentiated from the other cells of the tissue solely by Unna's method of staining. He finds them in various inflammatory affections of the skin, and thinks

they may be present in any form of acute inflammation with the exception of the suppurative. While he is uncertain about their origin, he denies that they are related to the connective-tissue cells.

Waldeyer at first believed that the cells described by Unna were identical with his plasma cells, but he afterward (39), in an article on the cells of the connective tissue, declares that the latter belong to the group of Mast-cells, which are different from the cells described by Unna. He does not think the word plasma cell should be used to designate any of the connective-tissue cells.

Marschalkó (40) has written an important article bearing on the presence and origin of these cells. He finds them not only in various tumors and chronic inflammatory conditions, but also in acute inflammation produced by the injection of carbolic acid into the liver, and in acute suppurative inflammations produced in the subcutaneous connective tissue. They appear in the tissue within twenty-four hours, and are derived from the lymphoid cells of the blood. These accumulate in the vessels in the periphery of the inflamed area, pass through the walls, and change into plasma cells in the tissues. He lays more stress on the morphology of these cells, the character of the protoplasm and nucleus, and the constant eccentric position of the latter, than on any peculiarity of staining. He finds them in the vessels of the liver at a distance from the inflammatory focus and in the vessels of the spleen. He also finds them in small numbers in the spleen, lymph-glands, and bone-marrow. He makes the very important observation that after the injection of tuberculin their numbers in the vessels of the spleen are greatly increased.

Hodara (41), working under the direction of Darrier, took up again the question of the presence of plasma cells in the normal tissues. He does not find them in the bone-marrow, lymph-glands, or spleen, in the normal embryo, child, or adult. He thinks that Marschalkó confounds the plasma cells with certain large mononuclear leucocytes, for which he proposes the name polyeidocytes, the name having reference to the variability of form which characterizes these cells. In some instances these cells may so resemble the plasma cells in size, form, character, and staining of the nucleus and protoplasm, as to be

indistinguishable from them. In general the difference between the polyeidocyte and the plasma cell consists in the great irregularity of form of the polyeidocyte and the variability in form, size, and staining of its nucleus. The only other article of importance concerning the plasma cells is that of Justi (42), who takes up the part which they play in the formation of the granulation tissue. Justi has included among the plasma cells large cells in the granulation tissue containing several nuclei. These cells are characterized as much by the character of the nucleus and protoplasm as by their manner of staining. Justi places the plasma cells among the leucocytes, and thinks they are derived from the lymphocytes.

It is perfectly evident that the cells we have described in the interstitial tissue, and many of those in the cell accumulations in the blood-vessels of the kidney, are the plasma cells of Unna. As to their origin, I hold the same opinion as Marschalkó, and they are derived from the lymphocytes. In the kidney they enter into the interstitial tissue by emigration from the blood-vessels. They may emigrate from the vessels as plasma cells, or they may be formed from emigrated lymphoid cells. They have been seen in the act of emigration, and the shapes of many of the cells in the interstitial tissue can leave no doubt as to their amœboid character. A great part of those in the interstitial tissue come from proliferation. It is difficult to understand why previous observers of these cells should have been so uncertain as to their mitotic division, and should have found it necessary to resort to the very uncertain theory of multiplication by direct division of the nucleus. Nuclear figures are as numerous in the interstitial cells, and as definite considering their size, as will be found in a rapidly growing carcinoma. Certainly there is no difficulty in explaining the numbers of these cells met with in the kidney by emigration and proliferation. Nuclear figures are also found in the cells within the blood-vessels, but not to the same extent as in the interstitial tissue.

With few exceptions, we have examined in every case of diphtheria and scarlet fever sections hardened and stained in the same way not only from local lesions, but from the liver, spleen, lungs, lymph-

glands, and bone-marrow. A detailed account of these examinations will appear later. It will be sufficient to say here that in nearly every case of both diphtheria and scarlet fever, but particularly diphtheria, both those in which interstitial lesions in the kidney were marked and where they were absent, great numbers of plasma cells are found in the spleen and bone-marrow. They are also found in the lungs, liver, capillaries, and lymph-glands. The principal seat of their formation seems undoubtedly to be the spleen, where they are formed from the lymphoid cells. Sections of the spleen in some cases show nearly the whole of every Malpighian body converted into plasma cells. Groups of them were also found in the pulp of the spleen and in the vessels. In all these places there are numerous nuclear figures. It is not probable, as Unna assumes, that direct nuclear division ever takes place in these cells. In the bone-marrow, which is red in nearly every case, numerous groups of plasma cells are also found. In the lungs they are found in great numbers in the blood-vessels, but especially in the connective tissue of the lung around the bronchi and blood-vessels. In places they play an important part in the cellular exudation in the focal pneumonia, but are found principally in the vessels and interstitial tissue, rarely in the alveolar exudation. In the lymph-glands, save in those in the immediate vicinity of the throat lesions, they are much less numerous than in the spleen and bone-marrow. In the liver they are in the capillaries, and probably come there from the spleen. Sections from the larger blood-vessels containing blood, and of decolorized blood-clot from the heart, are not so satisfactory for examination as the tissues, but they are found here in small numbers.

Investigations are now in progress to determine the presence and numbers of these cells in the peripheral circulation in cases similar to those in which they are found in the tissues.

With regard to the presence of the plasma cells in normal tissues, it is remarkable that those investigators who have taken up this question should have left out of consideration the organ in which they are always present in enormous numbers. In the alimentary canal, from the stomach to the anus, all the cells of the tissue between the epithe-

lium and the muscularis mucosa are practically plasma cells. They are comparatively rare in the follicles in this region.

In the tissues examined for fatty degeneration, both in fresh sections and after Flemming, there is no fat found in the plasma cells. Evidence of cell necrosis, as shown by nuclear detritus, is not found in them. The only evidence of degeneration which may be present in the plasma cells, both in those in the interstitial tissue and in those in the blood-vessels (Plate XXXVIII, Fig. 8), consists in the presence of vacuoles in the cells, or hyaline droplets which occupy the same position as to the vacuoles, and which often stain homogeneously with methylene-blue.

We are led to the belief that the plasma cells have their origin in the lymphoid cells from the similarity of their nuclei to those of the lymphoid cells, and from the presence of transitional forms between the lymphoid and plasma cells, as shown by the gradual accumulation of protoplasm of the same character as that of the plasma cell around the nucleus. This does not take place evenly around the nucleus, but always at one side. The constant eccentric position of the nucleus of the plasma cell is one of its marked characteristics.

In the examination of the kidneys of the autopsies in the last two and a half years I have found 42 cases of acute infectious diseases in which acute interstitial nephritis was present. The service in the hospital is an acute one, and there are comparatively few autopsies on chronic cases. All of the cases in which interstitial infiltration with plasma cells was found are included in the 42 cases; those in which only lymphoid cells were found around the large interlobular veins are not included. The majority of the interstitial cases come from the acute infectious diseases of children, which make up a large part of our autopsy material. In 103 cases of pure diphtheria a varying extent of interstitial change was found in 24. In 20 cases of pure scarlet fever interstitial lesions were found in 5. In 23 cases of mixed infection of diphtheria with scarlet fever, interstitial nephritis was found in 5, and in 2 out of 5 cases of diphtheria and measles. The other diseases in which the lesion was found were measles and whooping-cough, 1; empyæma, with subsequent diphtheria infection, 1;

lobar pneumonia and pericarditis, 1; epidemic cerebro-spinal meningitis, 1; lobar pneumonia, 1; acute glomerulo-nephritis, 1; acute endocarditis following abortion, 1.

Acute interstitial nephritis is far more common in the infectious diseases of children, and is occasionally seen in other acute infectious diseases. This fact and the focal character of the lesions suggest at once the agency of bacteria. The general diffuse degeneration of the epithelium which was so constantly found is evidently to be referred to the action of some soluble toxic substance. The results of the bacteriological investigation, both by microscopic examination of sections stained for bacteria and by cultures of the organs made at the autopsies, have not borne out the bacteriological theory. In all cases cultures were made at the autopsies from the primary lesions of the disease, whatever its character—from the blood, lungs, liver, kidneys, and spleen. The results of the cultures from the kidneys in the 42 cases of interstitial nephritis were as follows: In 24 cases of pure diphtheria the kidney was sterile in 6. In 11 the colon bacillus was found; in 1 case the staphylococcus aureus; in 5 cases the streptococcus pyogenes; in 8 the diphtheria bacillus, and in 1 the bacillus fœtidus. In the 5 cases of pure scarlet fever the colon bacillus was found in 2, the streptococcus in 3, and the staphylococcus in 1. In the 8 cases of mixed infection of diphtheria with scarlet fever or measles 2 were sterile; in 3 the streptococcus, and in 3 the colon bacillus was found. In the other 6 cases the colon bacillus was found in 3; the staphylococcus aureus in 2; the streptococcus pyogenes in 4; the pneumococcus in 1, and 1 case was sterile. I have included the colon bacillus in the result of the bacteriological examination, although I am not inclined to regard the presence of this as determined by cultures made at the autopsy as a matter of much etiological value. The result of the bacteriological examination of the kidneys in the same diseases, but in which interstitial lesions were not present in the kidneys, have shown the same results. The kidneys were sterile or the same organisms were present in about the same proportion. It is obvious from this that we cannot lay any weight on the presence of bacteria in the kidneys as a causative factor in the acute interstitial lesion.

In seeking an explanation of the focal character of these lesions their situation must be taken into account. They are most marked: First, in the boundary zone of the pyramids; secondly, beneath the capsule; and thirdly, around the glomeruli. There can be but two explanations for the presence of these foci. Cells are brought to the foci by the vessels, they emigrate from the vessels into the interstitial tissue, but probably the greater part of those in the interstitial tissue come from proliferation. Either there must be some physical condition in the circulation which may account for their accumulation in the vessels, or there must be some substance in the tissues which exerts a positive chemotaxis for just these cells, and which causes both their accumulation in the vessels and their emigration into the tissues. There is much which seems on purely physical grounds to favor the accumulation of cells in the long veins of the pyramids. These veins are long, they are numerous, and the circulation in them is slow. It is possible that the large cells in the circulating blood might be mechanically retained in these vessels. Weight is given to such a supposition by the fact that even small substances in the circulating blood may be mechanically retained here.

The hæmatogenous abscesses of the kidney, both those produced experimentally and those seen in man, are more apt to be formed in the upper part of the pyramid than elsewhere in the kidney. The organisms for the most part pass through the capillaries of the cortex; they become attached to the walls of the veins, and increase there, finally occluding the vessel and producing necrosis in the surrounding tissue. The presence of large numbers of these cells without any emigration speaks in favor of such a mechanical theory. The same would hold true, though to a less extent, in regard to the cell accumulations in the stellate veins. Occasionally the cells are seen here without interstitial lesions, but to nothing like the same extent as in the pyramidal vessels. Nuclear figures are found in the cells within the veins. The cells can pass through the vessels into the interstitial tissue, and the same conditions which led to their production and proliferation in the first place could cause them to proliferate in the interstitial tissue. Tumor cells can act in this way, and there is cer-

tainly some temptation to regard the interstitial foci very much in the same light as tumor metastases.

Such a mechanical theory fails to explain the interstitial foci around the glomeruli. Occasionally a few cells may be found in the vessels of the glomeruli (Plate XXXVIII, Fig. 9) without any interstitial lesions. Not only do periglomerular foci speak against the mechanical theory, but these cells are not found to the same extent in the vessels elsewhere, even in organs where the conditions of the circulation would seem to favor their presence. A few are almost constantly found in the capillaries of the liver, but their production in the spleen would explain this. They are found in the vessels of the spleen and bone-marrow, and in both of these places they are evidently produced. On the other hand, assuming that their presence in the kidney is due to some substance which exerts a positive chemotaxis for them, there are equal difficulties in an explanation. They are not attracted by simple degeneration and necrosis of tissue, as are the polynuclear leucocytes, and are not simply secondary to epithelial degeneration. Dr. Morse in a study of cantharadin poisoning (not yet published) has shown most extensive degenerative lesions, and even necrosis, without any change in the interstitial tissue and without leucocytes.

Ribbert (43) has recently published an interesting article in which he endeavors to explain the focal accumulation of cells in the kidney. In this article he calls attention to the locality of the foci of cellular infiltration and atrophy. He finds in chronic nephritis the lesions in the same situations which I find in the acute interstitial process. Ribbert explains this localization by the degeneration produced in the irregular tubules (Schaltstücke) by the concentrated toxic urine, and by the toxic urine passing directly through the thin capsules of the glomeruli. The pyramidal lesions are due in part to the toxic action of the urine in the collecting tubules, and in part to toxic substances which have passed into the tissue, passing along the lymph spaces to the large lymphatics in the boundary zone. It is possible that the general degeneration of the epithelium which we find in these cases may be due either to the toxic qualities of the urine or of the blood. The interstitial lesion may be due to some substance excreted by the

kidney, the foci being determined either by the action of this material where it becomes more concentrated, or where it escapes into the tissue, or the paths which it takes in the tissue. It is possible that the physical conditions of the circulation in the kidney may play a part, and possibly an important part, in determining the foci.

But little is gained from the clinical study of these cases. There was albumin in the urine, as there is in all cases of diphtheria. In none of the cases was there marked diminution in the amount of urine, and in none was there œdema. There was apparently nothing in the clinical history of the cases which called especial attention to the condition of the kidneys. In the literature of the disease there are some cases in which the clinical history is given. In Biermer's case, the disease being scarlet fever, the renal symptoms began two weeks before death, with marked diminution in the amount of urine, and dropsy followed by uræmia. In 3 cases of acute lymphomatous interstitial kidney in scarlet fever which Wagner reports, 1 died on the tenth and 2 on the seventeenth day of the disease. All 3 of the cases had pharyngeal diphtheria. Klein found the condition most frequently in those patients with scarlet fever who died after the ninth day of the disease. Friedländer found the lesions, which he describes as interstitial septic nephritis, in those cases of scarlet fever which are accompanied by severe diphtheritic septic processes.

The disease has a rapid course, and normal urine may be seen even twenty-four hours before death. Usually there is no œdema. It is probable that the 12 cases which Friedländer described, and which were from 229 autopsies on scarlet fever, represented marked types of the disease in which the lesions were evident macroscopically. Had he made systematic microscopic examinations he must have found minor degrees of similar lesions much more frequently. Sörensen gives the clinical histories of 3 cases. He also says the interstitial form is most common in cases of severe diphtheritic septic scarlet fever, and appears early in the disease. In one of his cases there was slight œdema and ascites.

In addition to these 42 cases, in which the interstitial lesions of the kidney were due to a general disease, 3 were found in which the interstitial process was due to the local action of bacteria.

The first case was one of ascending ureteritis and pyelonephritis. The kidneys were enlarged, weighing 390 grammes (small, slender woman), injected, somewhat œdematous, and on section whitish-yellow streaks in the cortex extended from the bases of the pyramids toward the capsule. The tissues in these areas of infiltration were not softened. Cultures from these areas gave colon bacilli and a few colonies of streptococci. On microscopic examination of sections in some of the larger areas there was a mass composed of necrotic tissue and filled with nuclear detritus. There were small areas where the necrotic tissue seemed to have been composed of pus cells, but for the most part the remains of the cells and nuclei seemed to have come from large cells. In the periphery of the larger foci, and in the whole extent of the smaller, there was intense infiltration of the tissue with plasma cells. Sections from the ureter and pelvis of the kidney showed slight superficial necrosis, comparatively few pus cells, and great numbers of plasma cells. Plasma cells were found in the vessels in the neighborhood of the foci.

The second case was one of hæmatogenous abscesses of the kidney following carbuncle of the neck and thrombosis of the lateral sinuses. The kidneys contained numbers of small abscesses, which were most numerous in the boundary zone of the pyramids. Cultures showed a general infection with staphylococcus aureus. Sections showed typical metastatic abscesses with masses of large micrococci in the centre, and around these a mass of pus cells with much nuclear detritus. In the tissue on the outside of the abscesses there were numbers of large epithelioid cells which often contain micrococci and other cells inclosed in them. Outside of these there was extensive infiltration, with plasma cells. Plasma cells were found in the vessels.

The third case showed both processes, a general interstitial nephritis and small abscesses with surrounding infiltration with plasma cells. This was from a case of scarlet fever complicated with extensive abscess-formation beneath the scalp. Cultures showed a general staphylococcus infection.

In all three cases the spleen was enlarged, and on section showed large numbers of plasma cells in the tissue.

SUMMARY.

Acute interstitial nephritis is found in the infectious diseases of children, particularly in diphtheria and scarlet fever, but may be met with in other infectious diseases.

The disease is characterized by general and focal infiltration of the interstitial tissue of the kidney with cells which correspond to those which Unna has described under the name of plasma cells. The focal character of the infiltration is marked; even in the cases in which all parts of the kidney show some interstitial cellular infiltration the cells are most abundant in certain foci. These foci are found in three places: in the boundary zone of the pyramids, in the subcapsular region of the cortex, and around the glomeruli. A considerable number of cases is found in which the blood-vessels of the boundary zone of the pyramids contain numbers of lymphoid and plasma cells without any infiltration of the interstitial tissue. The new cells in the interstitial tissue are due to emigration from the blood-vessels and multiplication by mitotic division of the cells which have emigrated. The cells can emigrate as plasma cells or as lymphoid cells, and the latter may change into plasma cells in the tissues. In the normal individual, plasma cells may be formed in the mucous membrane of the intestine, where they practically form the entire tissue between the epithelium and the muscularis mucosa, and to a limited extent in the spleen. In diphtheria, in scarlet fever, and probably in a number of infectious diseases, plasma cells are formed in great numbers in the spleen and bone-marrow, and to some extent in the lymphatic glands. In the spleen they are formed from the cells of the Malpighian bodies, which are often principally composed of them, and to some extent from the cells in the pulp. They are formed from the lymphoid cells.

No adequate explanation is found for the focal character of the lesions in the kidneys. There is some ground for believing that the physical conditions of the circulation may have something to do with their accumulation in the vessels in certain places. It is also possible that in the interstitial foci there may be soluble substances which exert a positive chemotaxis for them. Such substances may be found

in the urine, which may exert its influence on the interstitial tissue in different places. The explanation of the foci cannot be found in primary focal degeneration of the epithelium. Epithelial degeneration in these cases is always present, but it is diffuse. In foci where it is more intense and due to the interstitial changes, polynuclear leucocytes are found in the tissue, in the degenerated epithelium and in the tubules. Polynuclear leucocytes and not plasma cells are attracted by degenerated tissue. The foci are not due in these cases to the local action of bacteria.

In a number of the cases in which interstitial nephritis was found the kidneys were shown to be sterile both by cultures and by microscopical examination. In cases where bacteria were present they were found only in small numbers in cultures and not on microscopical examination, and their connection with the foci could not be demonstrated.

In three cases plasma cells were found in the interstitial tissue in definite bacterial diseases of the kidney. In these cases they were not found in connection with the lesions produced directly by the bacteria, but in the periphery of the purulent foci.

DESCRIPTION OF PLATES XXXVII AND XXXVIII.

PLATE XXXVII.

Fig. 1. Circumscribed focus of interstitial nephritis just beneath cortex. The large vein to the right of the specimen is a branch of the stellate vein. Zeiss AA, No. 2 ocular. All the other figures, with the exception of Fig. 3, are drawn with 1/12 Zeiss, ocular 2. All are drawn by camera.

Fig. 2. Plasma cells in interstitial tissue between tubules; the epithelium of the middle tubule is degenerated, the tubule contains polymorphonuclear leucocytes.

PLATE XXXVIII.

Fig. 3. Section of kidney magnified five times to show the granular appearance of the section, due to interstitial foci.

Figs. 4, 5, 6, and 7. Vessels in boundary zone, containing plasma cells. In 5 and 7 nuclear figures are seen.

Fig. 8. Vessel in boundary zone, containing two degenerated plasma cells.

Fig. 9. Section of vessel of glomerulus, showing plasma cells in vessel.

Fig. 10. Emigration of plasma cell from small vein in boundary line.

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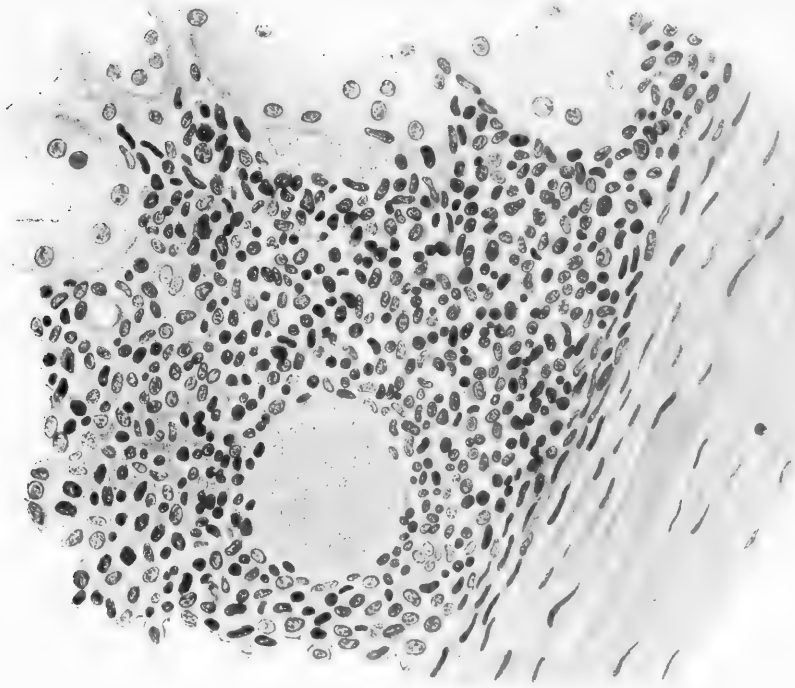

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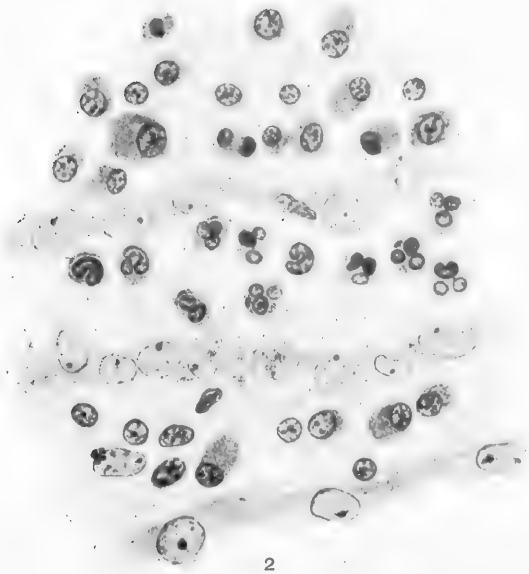

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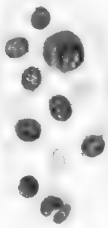

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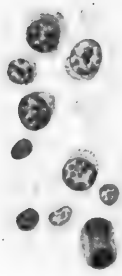

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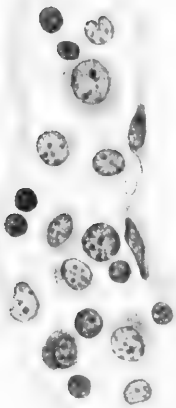

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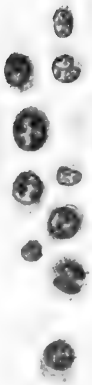

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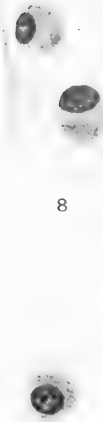

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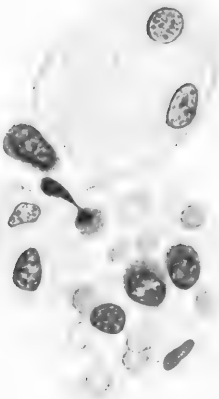

10

A CASE OF MYCETOMA (MADURA FOOT).

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PLATES XXXIX AND XL.

Mycetoma is a chronic inflammatory process, most commonly affecting the tissues of the foot, in which suppurative nodular swellings, sinus formation, and enlargement and distortion of the parts are prominent features. The bones may become involved and undergo a rarefying osteitis.

The affection is characterized by the presence in the diseased tissue and in the discharge from sinuses, of peculiar granules, usually not more than one millimetre in diameter, but sometimes larger. These granules in certain cases are of a black color, of irregular shape, and are hard and rather brittle. In general they resemble grains of gunpowder. In other cases the granules are whitish, greyish or yellowish in color and are of a soft or cheesy consistency. They have been compared to fish-roe in appearance. A very few cases are also recorded in which the granules were of a red color. There is no good evidence to show that more than one kind of granules are ever present in any one case of the disease.

On account of the fact that at least two very different kinds of granules are found associated with the lesions, two varieties or forms of the disease are recognized: the "melanoid" or black variety, in which the granules are black, and the "ochroid" or pale variety, in which the granules are white to yellow in color. A. Vandyke Carter, who was the first to show that mycetoma was a special pathological process and to name the disease itself and its varieties, considered the granules to be the cause of the affection and regarded them as representing stages in the life history of one and the same fungus. Carter claimed that he had cultivated this fungus and that it grew as a pink

mould. This pink mould was studied by H. J. Berkeley and named by him "Chionyphe Carteri." The connection between this pink mould and the granules was not clearly demonstrated, owing to the conditions under which the cultivation experiments were made, so that the pink mould has not been generally believed to represent the infecting organism in the process. There can be little doubt, however, that Carter saw and recognized in the black granules the same fungus elements which have been described by later observers. This is quite apparent from an examination of the plates in his monograph,* published in 1874.

It is now reasonably certain, however, that the organism of the black granules is of an entirely different character from the organism or organisms making up the pale or yellowish granules. Therefore in summarizing the results of various observers who have studied the nature of these granules, observations on the black granules will be considered separately from those upon the pale or yellow granules.

The disease is endemic in India and has been observed in Africa, Italy and other tropical or sub-tropical countries. In America it is of very rare occurrence, only three undoubted cases, in all, having been reported up to the present time. These are the cases of Adami and Kirkpatrick,† of Hyde, Senn and Bishop,‡ and of Pope and Lamb.§ These were all of the ochroid variety of the disease. In a case reported by Kemper and Jameson,‖ the diagnosis is open to serious question, so that it is not included in the list.

Observations on the granules of the melanoid variety. Bristowe¶ studied the black granules in a single case. He found them to consist "of densely aggregated ramifying tubules" embedded in a dark colored matrix. They frequently showed dilatation into round or ovoid cells. These structures he considered to be elements of a fungus identical with

* A. Vandyke Carter. On mycetoma or the fungus disease of India. London, 1874.

† *Trans. of the Assoc. of Amer. Physicians*, 1895; *Montreal Medical Journal*, January, 1896.

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¶ *Trans. Path. Soc.*, London, 1871.

that described by Carter. No cultivation experiments were made. Bristowe's description and drawings of the microscopical appearance of the granules are very satisfactory. It is quite evident that he had before him the same organism as that seen by Carter in the black granules.

Hogg * also examined a case of the melanoid variety, finding also fungoid structures in the granules, which, to judge from his description, were evidently the same as those described by Carter and by Bristowe.

In 1888 Bassini † described a case of the black variety which occurred in Italy. The granules were found to be made up of a mycelium of filaments imbedded in a dark brown matrix. These filaments were septate, 4 to 6 millimetres in diameter, and showed irregular swellings. They had a general radiate arrangement in the granule. Besides the granules Bassini noted the presence of strings or cords made up of the same fungus filaments imbedded in a brown matrix. No spore-bearing organs were found and no cultivation experiments were made. The mycelium was regarded as representing "an imperfect vegetative form of a fungus which, in a state of perfect evolution, would be furnished with a true reproductive apparatus." Leucocytes and young blood-vessels were noted in the tissues about the granules.

Kanthack ‡ studied three cases of the melanoid variety in microscopical sections. In all of these cases he found that the black granules seemed to consist, in some instances, of a structureless, olive brown, glassy or finely granular material, in which were imbedded hollow filaments of varying calibre, having a well-marked radial arrangement. Kanthack regarded these appearances as representing degenerative changes, and sought to show that the granules were made up of an organism closely related to actinomyces, as he had found in the case of the pale granules. It is manifest from his description, however, that he saw the same fungus mycelium in the black granules which had been noted by previous observers.

The latest and most satisfactory paper on the structure and nature of the black granules is that of Boyce and Surveyor.§ They studied seven cases of the melanoid form and found in all of them that "the black particles represent a curious metamorphosis of a large, branching, septate fungus." This fungus was imbedded in or surrounded by a brown pigment substance which was readily removed by "Eau de Javelle." The

* *Trans. Path. Soc.*, London, 1872.

† *Archiv. per le scienze mediche*, 1888, xii (1888), 309.

‡ *Journal of Path. and Bact.*, i (1893), 140.

§ *Trans. of the Royal Society of London*, 1894; *Proc. Royal Soc.*, London, 1893.

hyphæ showed great variations in the size and shape of their segments. They were branched and often disposed in a radiate manner at the periphery. No organs of fructification were observed. The fungus was not cultivated.

Observations on the ochroid variety. Carter's view that the pale particles represented a stage in the evolution of the same fungus as that making up the black granules has already been mentioned.

In 1870 Moxon and Hogg * examined a case of this variety. They found that the granules showed in some instances a texture of fine fibrils, but they were unable to come to any conclusion as to their nature.

Kanthack † examined histologically twelve specimens of the ochroid variety and states that "the parasite of the ochroid variety agrees morphologically and structurally with the typical and perfect ray fungus, and there can be no reasonable doubt that both belong to the same botanical group."

Vincent ‡ found that the granules in a case of the pale form of the disease were made up of a streptothrix, which grew upon potato with a marked red color. In a later paper published with Gémy,§ Vincent reports isolating in cultures the same streptothrix from a second case.

Hewlett || examined two cases of the ochroid variety and states that the granules were very like colonies of actinomyces in structure. He considered that the process was actinomycosis.

Boyce and Surveyor ¶ studied eighteen cases, and conclude that "the white particles consist largely of caseous material and of the remains of a lowly organized fungus, presenting in very many instances some of the characteristics of the fungus of actinomycosis." This organism they regarded as entirely distinct from that found in the seven specimens of the melanoid variety which they examined.

Boyce ** also reports having cultivated a streptothrix from a case of the ochroid variety. This streptothrix differed in its cultural peculiarities from other similar organisms.

Adami and Kirkpatrick †† conclude, from an examination of the case of the ochroid variety observed in Canada, that the granules "were

* *Trans. Path. Soc.*, London, 1870.
† Loc. cit.
‡ *Annales de l'Inst. Pasteur*, viii (1894), 129.
§ *Annales de dermatologie et syphilographie*, Paris, 3me sér., t. vii, Nov., 1896.
|| *Trans. Path. Soc.*, London, 1893.
¶ Loc. cit.
** *Hygienische Rundschau*, iv (1894), 529.
†† Loc. cit.

identical in general appearance with actinomyces, forming lobate, reniform masses with central dense mycelium and a radiate arrangement of filaments or clubs around the periphery. These clubs, however, were larger than those of actinomyces."

Bishop,* in the paper published with Hyde and Senn, gives the results of his microscopical examination of a case occurring in Chicago. The granules by direct examination were made up of a dense granular central portion and a more transparent peripheral portion in which an obscure radial striation could be observed. No clubs were observed at the periphery. In sections of the tissues the granules consisted of more or less rounded masses, often having a scalloped border and occurring singly and in groups. The central portions stained faintly with hæmatoxylin. Under a high magnifying power delicate filamentous threads, having more or less of a radial arrangement, were to be made out in the masses, and, at the margin of some, delicate radiating lines. There was much to suggest actinomycosis. The tissue changes consisted essentially in the formation of granulation tissue in which giant cells were present.

From a consideration of the results of this list of observers it is quite evident that the disease known as mycetoma is due to at least two different organisms, or in other words, that the black granules of the melanoid form are composed of an organism entirely different from the organism or organisms making up the pale or ochroid granules.

There can be little doubt that the melanoid granules are composed essentially of a fungus or a hyphomycete, and that probably one and the same fungus has been seen by all observers.

The chief thing lacking in these studies of the melanoid granules has been the growing of this fungus. This cultivation has been accomplished in the case reported below.

As to the nature of the granules in the ochroid form it is difficult to form an opinion in some of the cases. It seems clear that, in most of them at least, the granules are composed of organisms of the streptothrix group, to which actinomyces is now generally thought to belong, and not to be the more highly organized hyphomycetes. That some of the cases of the ochroid variety are nothing more or less than actinomycosis seems not unlikely.

* Loc. cit.

On account of the rarity of this disease in America, and because of the interest attaching to the parasite, the following case is reported:

Clinical History. The patient, an Italian woman, aged twenty-six, was admitted to the service of Dr. H. H. A. Beach, in the Massachusetts General Hospital, December 29, 1897. She was first seen in the Out-Patient Department by Dr. C. A. Porter, who recognized the condition as probably madura foot. The tissues of the left foot in the region of the base of the second and third toes on the plantar aspect presented a swollen appearance with desquamation of the epithelium. In the skin over this swollen area there was a small sinus, from which exuded on pressure a dirty, greyish fluid, containing some black, hard, irregular granules like grains of gunpowder. On the dorsum of the second toe, near its tarso-metatarsal joint, there was a small ulcer.

The disease was first noticed in July, 1897. The patient had lived in America several years. A piece of tissue was excised from the second toe for microscopical examination, and from the results of this, as well as from the appearance and character of the black granules associated with the condition, the diagnosis of mycetoma, or madura foot, of the melanoid variety was made. Amputation of the four toes, together with a part of the four corresponding metatarsal bones, was performed by Dr. Beach.

The dissection of the amputated part showed the following conditions: In the soft tissues of the plantar side of the foot near the tarso-metatarsal articulations and immediately beneath the skin was a pigeon's-egg sized, ovoid tumor nodule, sharply defined from the surrounding tissue (Plate XXXIX, Fig. 1). This nodule on section consisted of a soft, in places gelatinous, myxomatous-like tissue, traversed by an indefinite reticulum, which divided it into indefinite small areas; in these areas were imbedded black, irregular granules less than a millimetre in diameter, resembling grains of gunpowder. These granules occurred singly and in groups. In one or two places the tissue of the nodule was opaque and yellowish, as from necrosis. Two other similar nodules of small size, containing black granules, were also present. One was situated in the soft tissue of the dorsum of the foot near the base of the second and third toes; the other in the soft tissue of the first phalanx of the second toe. The larger of these nodules was about the size of a pea. The bones were not involved.

The structure of the black granules. Macroscopically the granules are of an irregular or mulberry shape, of a black or dark brown color, and usually less than one millimetre in diameter. They are sometimes bound together by shreds of tissue or of exudate. They are hard, rather brittle, and are not readily broken up under a cover-glass.

Microscopically they present the appearance of irregular, lobate masses of a dark brown or black opaque substance. Upon being broken up and examined with high magnifying power, little more than indefinite, brown, somewhat refringent masses can be made out. Occasionally structures suggestive of fungus may be seen, but there is little else to indicate a vegetable nature. They are very resistent to most reagents, but by soaking them for a few minutes, with or without heating, in the ordinary hospital solution of sodium hypochlorite, they lose their brown or black color and become more or less completely white and soft, according to the duration of the action of the reagent. A strong solution of potassium hydroxide also softens them to a certain extent, but does not bleach them so quickly.

Granules from the hardened specimen, bleached and softened as above outlined, when crushed and dissociated under a cover-glass, are found to consist of a mass of fungus structures together with more or less brown pigmented imbedding, or investing material, as described and figured by Boyce and Surveyor,* and others.

Under a high magnifying power (Plate XL, Fig. 7) the greater part of the mass of the granules is seen to consist of ovoid or rounded, translucent bodies of varying sizes, closely packed together, having homogeneous interiors or containing a few refractive granules. Among these bodies typical septate, branching hyphæ may be seen, sometimes showing dilatation or varicosities of their segments. The ovoid or rounded bodies seem to be dilated or degenerated hyphal segments, for not only may they be seen in continuity with the hyphæ, but transitions between them and the hyphæ with swollen segments may be made out.

The periphery of the granules has the appearance of being made

* Loc. cit.

up of closely set, radiating hyphæ, usually showing more or less swelling or degenerative changes in their segments.

No undoubted spore-bearing organs were observed, but occasionally a large spherical, thick walled body containing smaller rounded or ovoid or elongated refringent bodies was observed. These were regarded as representing some peculiar mode of growth or an "involution," rather than spores and spore-bearing organs.

In specimens treated with caustic potash solution, when the removal of the interstitial pigmentary substance is not complete, branched hyphal tubes may be made out imbedded in a brown refringent substance.

The test for hæmin in the black granules was negative.

In sections of the tissues, under the microscope, the granules are seen as rounded, lobate, reniform or irregular bodies of a more or less reticular structure in general (Plate XXXIX, Figs. 2 and 6, Plate XL, Fig. 8). They are deeply stained by the basic aniline dyes, but are not stained by hæmatoxylin.

In sections stained with the latter they appear to be chiefly composed of a brown-colored, somewhat hyaline substance, arranged throughout the main portion of the granules in the form of a reticulum and in indefinite or rounded clumps. This substance at the margin may merge into a narrow zone of yellow, very hyaline refractive material, in which more or less of a radial striation may sometimes be apparent.

The results of the study of the granules in sections stained in various ways may be summarized as follows:

The granules, as a rule, are made up of a central portion and a cortical portion or rind, without any definite separation between them. In the central portion are usually branching trabeculæ and rounded masses, composed of a hyaline substance, in the midst of which are numerous structures like fungus elements, cut in various planes. In some instances the central portion is made up mainly of an amorphous or indefinite material with only a few fungus hyphæ. In general the central portion is less dense in structure than the cortical portion or rind. The latter in most cases consists of a hyaline material, con-

tinuous with the hyaline trabeculae of the central portion, in which fungus elements are imbedded, sometimes arranged more or less in a radiate manner, sometimes not. The margin is irregularly scalloped or dentated. In some cases the cortical portion seems to be made up of more or less atypical hyphæ and elongated processes of hyaline substance radially disposed. In a very few instances the granules appear in the sections as irregularly shaped masses of homogeneous, hyaline or fine granular material, in which unstained or empty fungus tubules or hyphæ are embedded (Plate XL, Fig. 8).

In brief it may be said that the granules are composed of a mycelium of hyphæ or fungoid elements, more or less degenerated, embedded in or surrounded by a hyaline brown-colored refringent substance, which itself forms more or less of a reticulum.

The Gram stain and the Weigert fibrin stain, or modifications of them, stain the substance of the granules, to a variable extent depending on the amount of the decolorizing agents applied. The marginal portions of the granules hold the stain longest. In only a very few instances could the hyphæ be differentially stained by these methods.

Cultures. About sixty-five of the black granules were planted in various culture-media after having been washed in sterilized bouillon to remove contaminating organisms as much as possible. Nearly all of these granules were taken from freshly cut portions of the nodules of the foot after amputation. From approximately twenty-five of the sixty-five granules, a growth of a hyphomycete was obtained. The original cultures, with one or two exceptions, showed more or less contamination with the staphylococcus albus, which was abundantly present in the diseased tissue. The exceptions were in the cases of one or two grains obtained from the sinuses before the amputation and after peroxide of hydrogen had been used at intervals in the sinuses. This reagent had probably destroyed most or all of the pus cocci adherent to the grains, so that the washing in sterile bouillon sufficed to render it free from living contaminations.

The growth in all cases began from the grain and appeared after about four or five days or later. On solid culture-media it first appeared as a tuft of delicate, whitish filaments springing from the black

grain. These in the course of a few days increased in number and length, and in the case of the potato formed a dense, whitish or pale brown felt-work or membrane, having a tendency to spread widely (Plate XL, Fig. 12).

Granules planted in potato infusion, in which the contaminating cocci grew only feebly, and granules obtained free from contamination and planted in bouillon, showed after four or more days a faint greyish halo about their peripheries. In the course of some days this increased in size and was seen to consist of long, delicate, radiating filaments (Plate XL, Fig. 9), which grew in length until they reached the wall of the tube and produced the appearance of a powder puff-ball, with the granule in the centre (Plate XL, Fig. 11). Granules were readily obtained free from bacterial contamination and planted in bouillon for development, by selecting those grains which, after remaining on solid culture-media for several days, were well separated from colonies of cocci. Many of the granules, *i. e.* about forty out of the sixty-five, failed to develop any fungus. This fact bears out the idea that many of the fungus elements in the granules are degenerated, as has been mentioned.

The character of the hyphomycete may be summed up as follows:

Morphology (Plate XL, Figs. 8 and 10). Long branching hyphæ from 3 to 8 μ in diameter. In the younger ones delicate transverse septa are present. In the older forms there may be swellings at these points, and the hyphæ may appear as a string of oval-ended, plump segments. The filaments have a definite wall and in their interior, granules or pale areas may be seen. The branching occurs by the formation of lateral outgrowths or buds. No spore-bearing organs were observed.

Potato. It forms a dense, widely spreading, coherent membrane, or layer, of velvety surface, pale brown in color throughout its central portions, and white at its peripheral portions. A marked feature is the appearance of small spherical globules or droplets of dark, coffee-colored fluid on the surface of this layer. The potato takes on a dark brown color and becomes very moist (Plate XL, Fig. 12).

Bouillon. The growth always proceeds from the planted material,

whether grain or fragment of mycelium, in the form of fine radiating filaments, and soon produces a puff-ball appearance (Plate XL, Figs. 9, 11). Eventually the whole area of fluid is filled up with the radiating mycelium and a definite mycelial layer is formed at the surface. The medium acquires a deep coffee-brown color.

Potato Infusion. This was prepared by boiling about 20 grms. of potato in water, filtering and making up to 1000 cc. without neutralizing, as used by Vincent.* In this the growth proceeds as in bouillon, except that no surface layer is formed. A peculiarity, however, noted in old cultures consists in the appearance of numerous black granules, about 1 mm. or less in diameter, in the midst of the mycelium.

These granules consist of closely packed spherical or polyhedral cells, together with some short, thick, segmented hyphæ. The walls of these cells have a black appearance and masses of them are black and opaque under the microscope. They seem to represent masses of interlacing hyphæ whose segments have been much shortened and widened, and otherwise changed.

These black masses were kindly examined by Prof. W. G. Farlow of Harvard University, who considered them to be "sclerotia."

Agar-Agar (plain and glucose). The growth appears as a meshwork of widely spreading filaments, of a greyish color, on the surface of the medium. In old cultures black "sclerotia" are found as in the potato infusion. In stab cultures growth appears only at the surface.

No results were obtained from the inoculation of animals with the original granules or with cultures.

Pathological Histology. The tissue composing the nodules consists essentially of a formation of more or less atypical connective tissue, in various stages of development, in which foci of suppuration are present in association with the granules.

Some of the granules lie in small cavities containing polynuclear leucocytes, loose epithelioid cells and cellular detritus. These cavities may be lined either by a wall of vascular granulation tissue or by masses of epithelioid cells, together with multinucleated giant cells

* Loc. cit.

(Plate XXXIX, Figs. 2 and 4). Other granules are closely invested by a zone of epithelioid and giant cells, and outside of this there may be an infiltration with lymphoid and plasma cells. The nodule thus formed about the granule resembles very closely a tubercle in structure. This tubercle-like formation is shown in Plate XXXIX, Figs. 5 and 6. The giant cells are often of large size and may have a peripheral arrangement of their nuclei. They are a very prominent element in the lesions.

The primary effect produced by the parasites upon the tissues seems to be the development of nodules of epithelioid cells and of giant cells from the tissues immediately about them. Later, suppurative processes occur in the nodules and abscesses are formed, which in time give rise to the development of granulation and connective tissue in large amount.

The writer is under obligations to Dr. H. H. A. Beach, of the Staff of the Massachusetts General Hospital, and to Prof. W. G. Farlow, of Harvard University, for courtesies shown him in connection with the study of this case.

The photographs which accompany this paper were made by Mr. Louis F. Brown. For valuable instruction and suggestions in their preparation the writer is greatly indebted to Mr. John G. Hubbard, of Brookline, Mass.

DESCRIPTION OF PLATES XXXIX AND XL.

All of the figures are photomicrographs from original specimens.

PLATE XXXIX.

Fig. 1. Portion of amputated part showing the black granules and general character of the lesions as seen with naked eye.

Fig. 2. Section showing black granules and general features of the lesions as they appear under a low magnifying power—Zeiss a_2.

Figs. 3, 4, 5 and 6. Sections showing the granules and surrounding cells. All, with the exception of Fig. 4, taken with Zeiss apochromat. 16 mm. Fig. 4 taken with Zeiss apochromat. 4 mm. Figs. 5 and 6 represent the tubercle-like nodules, with the parasite in the centre, as described in the text.

PLATE XL.

Fig. 7. Fungus elements from a granule after bleaching and softening with sodium hypochlorite solution. A branching, septate hypha is shown. Zeiss apochromat. 4 mm.

Fig. 8. Section showing margin of two contiguous granules, highly magnified. Zeiss apochromat. 2 mm.

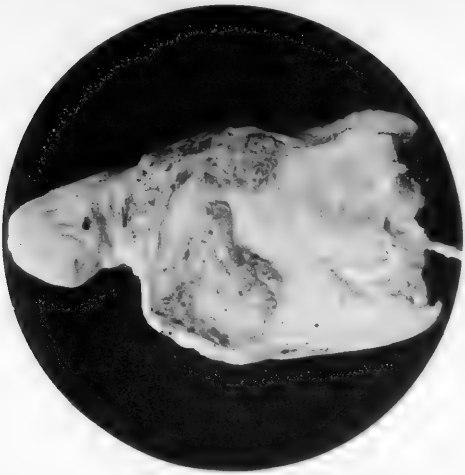
The small clearer areas represent fungoid elements. The darker substance represents the hyaline imbedding substance.

Fig. 9. Black granule with mycelial growth proceeding from it. Zeiss a_2. Culture in bouillon.

Fig. 10. Showing structure and appearances of the hyphæ of the mycelium obtained from the granules. Zeiss apochromat. 4 mm.

Fig. 11. Two bouillon cultures showing the powder puff-ball appearance. In one the black granule is seen in the centre of the growth.

Fig. 12. Potato culture of the hyphomycete obtained from the granules. The black globules are composed of a dark brown fluid.



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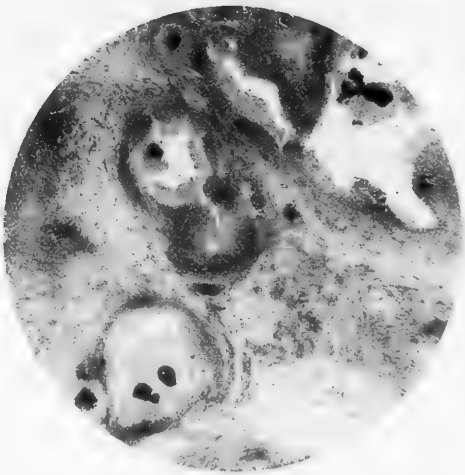

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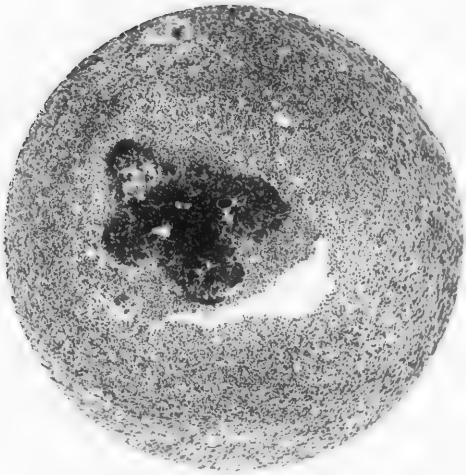

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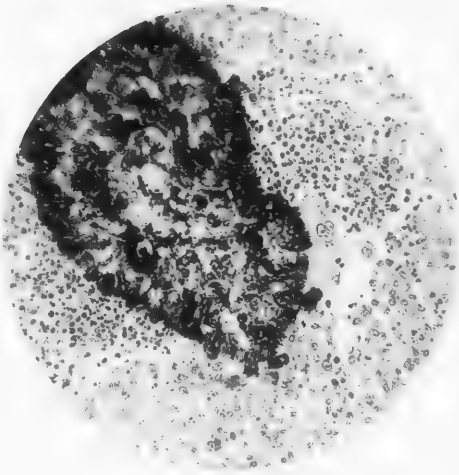

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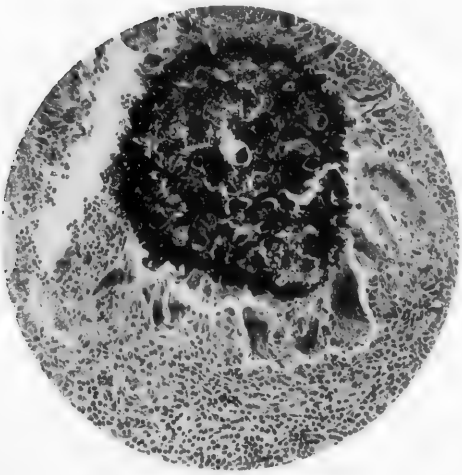

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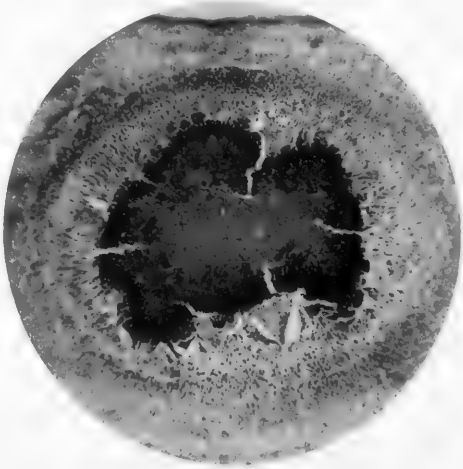

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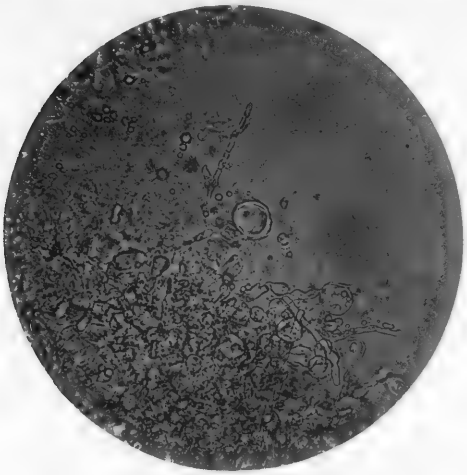

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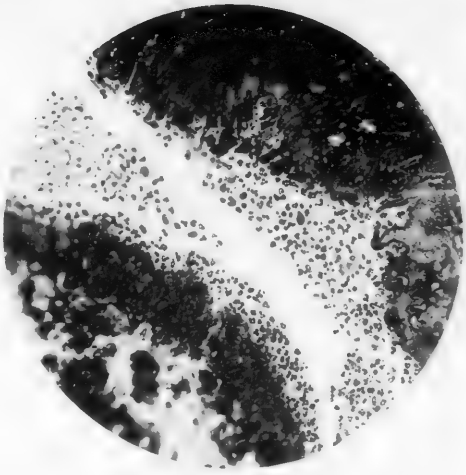

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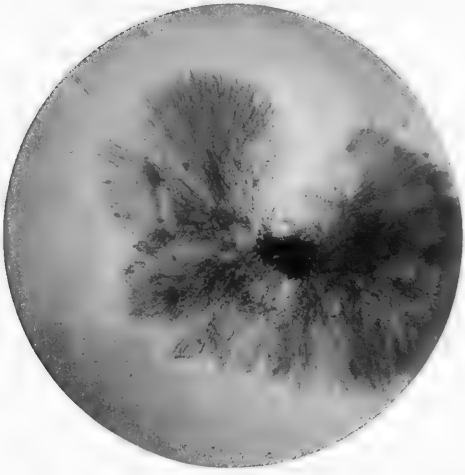

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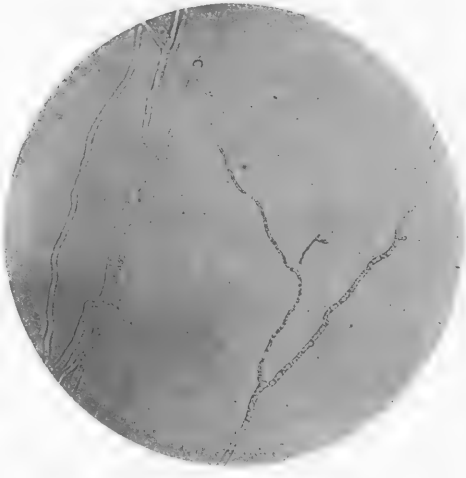

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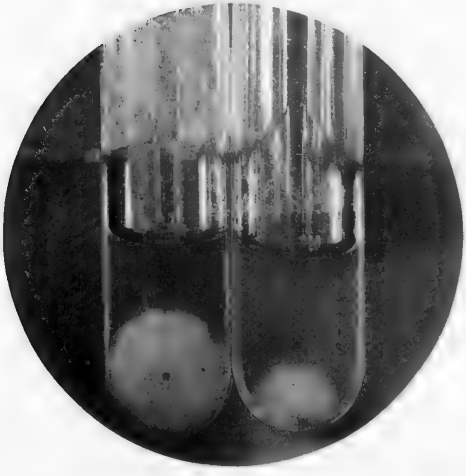

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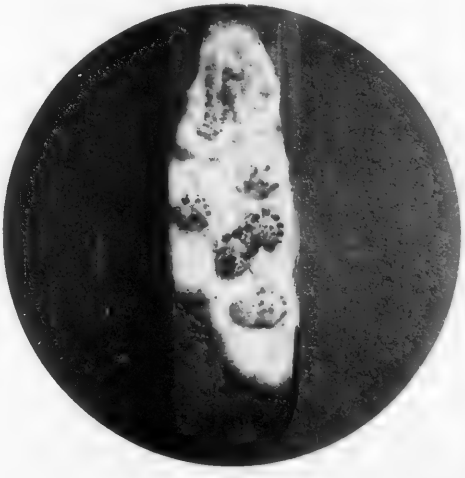

12

PSEUDO-TUBERCULOSIS HOMINIS STREPTOTHRICHA.

By SIMON FLEXNER, M. D.

(*From the Pathological Laboratory of the Johns Hopkins University and Hospital.*)

PLATE XLI.

The researches of the past quarter of a century have brought to light many species of micro-organisms whose relations to certain pathological processes have been shown to be of the nature of cause and effect. Among these are included representatives from various groups, bacteria, protozoa, yeasts and moulds. The greatest success thus far achieved has been in the study of the bacteria; indeed, until recently these were regarded as almost the only vegetable micro-organisms capable of causing infectious pathological processes. Our knowledge of the biology of the pathogenic vegetable micro-organisms has more recently been extended in two directions. In the first, certain of the blastomycetes have been proven to be pathogenic for man and animals; in the second, the morphology of the bacteria has been found to depart from the simple formula originally assigned to them. In view of these facts it has been considered necessary to designate a new class for the reception of those vegetable micro-organisms which, while exhibiting affinities with the bacteria, at the same time possess certain features in common with the hyphomycetes. To this class Kruse has given the name *Streptothrices*. Its limits are ill-defined, chiefly for the reason that occasionally one of the common bacterial forms (B. tuberculosis, B. diphtheriæ, B. proteus) will show certain of the characteristics of the group, while for the most part they appear as simple rods.

The streptothrices resemble the moulds in that they develop from spores into cylindrical, dichotomously-branching threads, which alternately grow into colonies the appearances of which suggest a mass of radiating filaments (mycelia). Certain of the threads become fruit-hyphæ, and these break up into chains of round spore-like bodies.

With the bacteria they agree in so far as they lack a doubly-contoured membrane, in not being composed of hyphæ filled with fluid and granular contents and separated from each other by partition walls, and from the fact that they appear as homogeneous threads, resembling the filiform bacteria, which, finally, in older cultures, separate into short bacillary and coccus-like structures (Kruse).*

The researches of Boström † prepared the way for the later studies upon the micro-organism causing actinomycosis and led finally to its classification with the streptothrices under the name of streptothrix actinomyces (Rossi-Doria). Another pathogenic species is represented by streptothrix maduræ (Vincent). These two are the best known pathogenic forms; and an extensive study of their characteristics shows that it is not improbable that they may represent genera rather than species, so that ultimately it may become necessary to distinguish different species or, perhaps, only varieties of each.

The remaining streptothrices described in connection with pathological processes in man are much less well defined. All the pathogenic possibilities of the class have not as yet been discovered. Rosenbach ‡ found a branching micro-organism, capable of growing upon artificial media and of reproducing upon inoculation a similar pathological condition, in an affection known as *erysipeloid* (Erythema exsudativum multiforme). Eppinger § isolated from a brain abscess, which had ruptured into the lateral ventricle and set up meningitis cerebrospinalis, a branching micro-organism which he regarded as a cladothrix (C. asteroides), but which by Kruse is classed with the streptothrices (S. eppingerii). Eppinger injected cultures into guinea-pigs and rabbits and observed that it caused a typical pseudo-tuberculosis. Indeed, the cerebral abscess suggested the disintegration of a large tuberculous focus. The case reported by Garten || is not so clear.

* Kruse, In Flügge's Die Mikroorganismen, 1896, ii, 48.

† Boström, Untersuchungen über die Actinomykose des Menschen, Ziegler's *Beiträge*, ix, 1890.

‡ Rosenbach, Ueber das Erysipeloid, *Archiv f. klin. Chirurgie*, xxxvi, 346.

§ Eppinger, Ueber eine neue, pathogene Cladothrix und eine durch sie hervorgerufene Pseudotuberculosis (cladothrichica), Ziegler's *Beiträge*, ix, 287.

|| Garten, Ueber einen beim Menschen chron. Entzündung erregenden, pleomorphen Mikroben. *Zeitschr. f. Chirurgie*, xli, 257.

This author obtained in pure culture a branching micro-organism from abscesses and a fistulous tract, extending from the upper dorsal vertebræ to the sacrum, the ribs and vertebræ showing erosions. While he regards the organism as a new species, its distinction from streptothrix actinomyces is by no means clearly established.

Additional instances of streptothrix infection are reported by Sabrazès and Rivière,* and by Ferré and Faguet.† The first case of Sabrazès and Rivière occurred in a man in whom abscesses were found in the brain, lungs and kidney (softened infarction). The pus from the brain abscess showed single and radiating, branching threads; in the kidney the filaments were much shorter, while in the lungs neither form was discovered. In cultures cocci were found associated with the streptothrix, the latter growing anaerobically only. In a second case these observers isolated from sputa and from a subcutaneous abscess in a man an ærobic streptothrix, pathogenic for small animals. Ferré and Faguet obtained in pure culture from an abscess of the brain in an epileptic, a streptothrix which proved, however, to be non-pathogenic for guinea-pigs and rabbits.

It is now well known that under certain unusual conditions the bacillus tuberculosis is capable of appearing in an irregular branched form.‡ Further observations are, however, necessary before accepting as final the results obtained by Friedrich § and Babes and Levaditi,‖ who inoculated cultures of the bacillus tuberculosis into the carotid artery and beneath the dura mater, respectively, in rabbits, and found that the bacilli had not only branched but had also developed into rosettes resembling the "Drusen" of actinomyces.

Another streptothrix pathogenic for man is one upon which a pre-

* Sabrazès et Rivière, Sur un streptothrix rencontré dans un cas d'abcès du cerveau et d'infarctus suppuré du rein. *La presse médicale*, 1894, Sept. 22.

† Ferré et Faguet, Streptothrix et abcès du cerveau, *Mercredi médical*, 1895, 441.

‡ For the literature on this subject see Craig. The branched form of the Bacillus tuberculosis in sputum. *Journal of Experimental Medicine*, iii (1898), 363.

§ Friedrich, Ueber strahlenpilzähnliche Wuchsformen des Tuberkelbacillus im Thierkörper, *Deutsche med. Wchnschr.*, xxiii, 1897, 653.

‖ Babes et Levaditi, Sur la forme actinomycosique du bacille de la tuberculose, *Arch. de méd. exper.*, 1897, 1041.

liminary note appeared in the Johns Hopkins Hospital Bulletin * for June, 1897, and of which the present paper will contain a more complete account.

A consideration of the morphology of the peculiar micro-organism, which was associated with consolidation, necrosis and early cavity formation in the lungs of a human being, and the nature of the pathological process, which in so many ways suggested the caseous pneumonia of true tuberculosis, led to the adoption of the name *Streptothrix pseudo-tuberculosa* for the organism, and *pseudo-tuberculosis hominis streptothricha* as a designation for the pathological condition.

The clinical history of the case offered nothing of especial importance. The patient was a male, colored, aged 70 years, in whom extensive consolidation was made out in both lungs. The symptoms were broadly those characteristic of pulmonary tuberculosis. Sputum was carefully watched for during his stay in the hospital, in Dr. Osler's service, but none was obtained. No microscopical examination could therefore be made.

The autopsy was made 19 hours after death, the body in the meantime having been kept on ice. No evidences of post-mortem decomposition were noticeable. The description of the viscera is limited here to the organs which exhibited pathological alterations.

The *lungs* are voluminous and meet in the middle line anteriorly. They are not bound to the chest wall. *Left.* All of the upper lobe, except the anterior edge, is consolidated more or less perfectly. Where the consolidation is complete, as in the apical portion, the lung presents an opaque appearance, and is grey in color; beginning softening (disintegration with early cavity formation) is going on. These cavities often still contain the products of disintegration, and all appearances of reactive encapsulation are wanting. The lower zone of this lobe is more flaccid and contains scattered areas of consolidation between which the lung tissue is œdematous and swollen, although not completely airless. The lower lobe is congested and œdematous; it contains a number of scattered and partly calcified nodules which on an average are the size of a split pea. The pleura over the hepatized area is covered with a fibrinous exudate. *Right.* The middle lobe is almost completely consolidated. The other lobes contain several small calcified nodules

* Flexner, Pseudotuberculosis Hominis Streptothricha, a preliminary note. *Johns Hopkins Hospital Bulletin*, vii, 1897, 128.

and a number of recent, tolerably circumscribed, caseous areas, averaging in size that of a walnut and surrounded by œdematous, imperfectly consolidated and dark lung substance. The lower lobe contains an almost globular, infiltrated area, 4.5 cm. in diameter, caseous in appearance, the centre of which is undergoing disintegration. The pleura over the fresh foci of consolidation is covered with fibrin and studded with small hæmorrhages.

Peritoneal Cavity. The intestines are moderately distended. The omentum is rolled up and thickened; it occupies a position beneath the transverse colon and extends across the abdominal cavity. The pelvis contains about 15 cc. of fluid of a brownish color and mucilaginous consistence. Between the intestinal loops delicate threads of fibrin exist. In addition smaller and larger nodules, resembling tubercles, usually translucent, are scattered irregularly over all the exposed peritoneal surfaces, and occur more uniformly upon and within the thickened, rolled-up omentum. The liver and spleen on section show similar nodules. The mesenteric glands are not perceptibly enlarged, but upon section minute, opaque tubercles are visible in them.

Histological Examination. The microscopical study of the tissues of the case embraced all the organs. Reference will be made here only to the lungs, the peritoneum and the omentum, in which organs the chief lesions existed.

The pathological process in the lungs consists chiefly of an exudation, which is not, however, of uniform character. For the most part the exudate is cellular, but in certain situations it is composed largely of fibrin.

A stained section of the lung macroscopically presents a variegated appearance due to this heterogeneous exudate. Where this is cellular, a deep nuclear staining is evident; elsewhere the tissue appears pale. The cellular exudate is intra- and inter-alveolar; it is rare, indeed, that the alveolar walls are free from infiltration. The cells composing it are chiefly polymorphonuclear leucocytes, but what is most striking is the extent of fragmentation which these cells have suffered. A small amount of fibrin exists among the cells. Many alveoli contain fibrin almost to the exclusion of leucocytes; this fibrin is dense and compact and encloses cells of the epithelioid type. In certain situations, especially near the somewhat thickened pleura, the

fibrin in the alveoli is undergoing rapid organization. The pleura is covered by a fibrinous membrane 3 or 4 times the normal thickness, capped by a thin line of dense fibrin in process of organization.

There is a marked tendency for the tissue showing the cellular infiltration to break down, with formation of cavities showing softened contents. It is found that this softening has taken place only where the infiltration of the alveolar walls has reached a high degree. But the gross examination of the lungs gave no adequate idea of the extent of this process of disintegration, many of the foci being microscopical in size. The disintegrating tissue differs from the general cellular infiltration only in containing more nuclear fragments and irregularly staining detritus. In specimens stained with hæmatoxylin and eosin this irregular material takes on a peculiar, imperfect hæmatoxylin tint, resembling that shown by certain kinds of caseation in tuberculous forms of pneumonia. In the regions in which the exudate is purely or largely fibrinous no such tendency to softening and disintegration is manifest. There is no attempt at limitation or encapsulation of the areas of cavity formation by a growth of demarcating connective tissue.

Circumscribed nodules having the size, form and appearance of miliary tubercles occur in small numbers. They are composed chiefly of epithelioid cells, some of which are disintegrating. Giant cells are not seen.

The bands of interlobular tissue are thickened by a new growth of tissue, and islands of fibroid tissue appear here and there in the lung substance. The blood-vessels are extensively obliterated. This obliteration is caused either by an endarteritis or by thrombosis; sometimes both processes are met with in the same vessel.

Of the structures contained within the abdominal cavity, the intestine and omentum more especially were subjected to histological study. Of these the latter gave the more interesting results. The thickening of the omentum is caused chiefly by a growth of tubercle-like nodules, which cover its surface and also occupy its interior. The nodules are, on an average, larger than miliary tubercles, and still larger masses are formed by the coalescence of several such foci.

In general two kinds of nodules are distinguishable. The larger, probably the more numerous, exceed in size a military tubercle; they are composed of a central mass of disintegrated or disintegrating cells and nuclei. Very irregular nuclear forms are met with, some of these doubtless being emigrated polymorphonuclear leucocytes. Nuclear figures occur now and then among the degenerating cells, but these are taken to be examples of karyorrhexis and not as representing true cell division. Definite fragments of nuclei are common. The centre of such a nodule is occupied often by a transverse section of a small vessel in which white blood-cells can be seen now and then; the lumen is lined by endothelial cells, usually in increased numbers. Beginning at the thin outer wall and often taking in everything except the endothelial cells, a homogeneous material may be noticed, which extends for a variable distance into the tubercles proper, obliterating cell-boundaries as it spreads. With Weigert's fibrin stain this substance takes on a distinctly blue color. The more peripheral parts of the nodules contain more unaltered cells, these presenting the usual appearance of epithelioid cells, although more elongated types (fibroblasts) and irregular leucocytes are also met with. The most external cells are lymphoid, and these, together with more or less polymorphonuclear leucocytes, go over into the surrounding adipose tissue and coalesce with other nodules. Giant cells appear rarely in the less degenerated parts.

In the sections stained with hæmatoxylin and eosin a delicate fibrillated network can be made out among the cells and fragments, which, in specimens stained especially for it, is found to consist of fibrin.

The smaller nodules differ from these in that they are made up of cells which are well preserved and possess large nuclei of the epithelioid type. The cell protoplasm is relatively abundant, and a suggestion of fibrillation is visible in the substance between the cells. This kind of nodule is found in the peritoneal covering of the intestines, where they form an almost uniform layer. Almost no degenerated cells are met with in these formations.

Giant cells are present within the nodules, but they are not abund-

ant there, and they occur without these in the more diffusely infiltrated tissues. Their forms correspond with those found in ordinary tubercle, showing as a rule the peripheral disposition of nuclei and the central hyaline protoplasm. But multinucleated giant cells either with peripheral or centrally massed nuclei occur with especial frequency within the lumina of small blood and perhaps lymph-vessels. It sometimes happens that such a cell occupies the central vessel in one of the first nodules described; but it is more common for them to appear in somewhat larger vessels outside or just at the periphery of the nodules. They are derived from the endothelial lining of the vessels which is met with in the several stages of proliferative activity leading to their formation.

The bacteriological examination. The examination for bacteria consisted in the study of cover-slips from the fresh lungs, the inoculation of glycerine-agar tubes, and the subcutaneous injection into a guinea-pig of a suspension from the consolidated lungs. After hardening the tissues, sections from the lungs, from the peritoneal covering of the intestines and from the omentum were stained for bacteria. Cover-slips from the lungs stained by Gabbett's method showed no micro-organisms which resembled the bacillus tuberculosis in their morphology, but there remained faintly stained in carbol-fuchsin upon the cover-slips numerous examples of a branching organism occurring often in clumps or convoluted masses, among which no ordinary bacillary forms were discovered. Through the use of Weigert's method of staining bacteria the micro-organism was rendered suitable for more exact study.

The cultures from the left pleural cavity and the peritoneum remained sterile. Three separate sets were made from the lungs. In all these, at the end of 24 hours, a vigorous growth of a bacillus identified as belonging to the group of B. coli communis had taken place. The streptothrix did not grow.

The guinea-pig showed no reaction to speak of at the site of inoculation, the adjacent lymph glands could not at any time be felt; the animal, however, lost in weight and died at the end of the 9th week, at the autopsy showing great emaciation. None of the lymphatic

glands were found enlarged; there were no lesions resembling tubercles in these and other organs, and cultures upon glycerine-agar, made from several sources, remained sterile. Cover-slips from the serous cavities, blood and viscera were negative for any kind of bacteria.

The further study of the organism stained upon cover-slips prepared from the lungs at the time of the autopsy, by Weigert's method, in which carbol-fuchsin is substituted for gentian-violet, shows it to possess the following characteristics: The micro-organism appears only in streptothrix forms, rarely as single branching filaments, but usually as masses, more or less convoluted, in which the several individuals seem at times to participate. This tendency to form convoluted masses renders the length of the filaments variable; some, however, stretch across the field of the microscope (1/12 objective). The thickness varies also, but to a less degree and in general is about the same as that of B. proteus. The branches are often short and rarely capped at the extremity with a conical swelling (conidia?). That these bodies are not actual conidia is, I think, further proved by their occurrence in the course of the filaments. They doubtless belong to the chromatic particles to be described later. There is protoplasmic continuity between the parent filament and the branch. The contours are slightly irregular, the staining sometimes not quite uniform, and very rarely just before its termination a filament will show 2 or 3 cross partitions suggesting the breaking up into as many short rods. Coccus-like forms were not encountered.

An irregularity in staining is one of the common features of the organism, an appearance, moreover, that depends in part upon the extent of the decolorizing process. The more deeply-stained filaments are a little thicker than those which stain faintly, and the chromatic granules or particles which lie close together in the former may be separated in the latter by widths of unstained protoplasm of greater diameter than the stained particles. In the most pronounced cases the chromatic bodies appear to lie within a non-staining homogeneous membrane (Plate XLI, Fig. 1). The possibility that this difference may depend upon other causes, *e. g.* degeneration, cannot be excluded, but the impression obtained is that it is more or less artificial, and has something to do with the degree of staining, etc.

Sections from several different parts of the lungs were stained first in hæmatoxylin and then in carbol-fuchsin, which was followed by pure aniline oil, for the purpose of differentiation and dehydration. The nuclei appear blue; the micro-organisms red. The latter are very abundant. Their relation to the pathological process is very intimate and somewhat as follows: The only parts of the lung tissue from which the micro-organisms appear to be absent are the areas occupied by the fibrinous exudate. The cellular exudate everywhere shows them, but the streptothrix is most abundant, appearing in the largest masses in the areas of rapid disintegration (Plate XLI, Figs. 2 and 3). The masses of the organism are somewhat larger than are met with in the cover-slips and the branching is more perfect, perhaps because the normal relations are less disturbed in the sections. Rarely, a small fragment of a filament or a separate thread will be present in a mass of pus cells and fragments of cells. The streptothrix appears, but in relatively small clumps, within the tubercle-like nodules; more often indeed a single branching filament is met with in these formations.

The staining of the threads is somewhat more irregular than in the cover-slip preparations; they present a granular, almost beaded appearance, and are thinner than under the former conditions (Plate XLI, Fig. 3).

The omentum especially, of the abdominal organs, was studied for micro-organisms. A large number of sections were treated first with carbol-fuchsin in the thermostat and then carefully decolorized with 1 per cent hydrochloric-acid alcohol. Bacteria were never found in these specimens. Other sections were then stained by the method used for demonstrating the streptothrix in the lungs. Almost every specimen prepared in this way showed bacteria, and often in very considerable numbers. They were contained within the tubercle-like nodules.

The forms of micro-organisms met with in this situation are simpler than those in the lungs. So far as could be discovered from many sections, the individual organisms never branch. They appear as rods of unequal lengths and of a curved or angular form. The staining is irregular, a beaded appearance being very common. The "bead-

ing" is quite irregular; more so, I am disposed to think, than is the case with B. tuberculosis. In examples showing the most pronounced irregularity, the organism appears as a nearly colorless filament with uneven deeply-staining red dots placed at different intervals from one another. In length as well as in width the rods rather exceed the average dimensions of the tubercle bacillus. It must, however, be admitted that not a few individuals could not be distinguished by their size alone from this micro-organism.

From the foregoing description there can be, I think, no reasonable doubt that the micro-organism encountered in the lungs differs widely from the known forms of B. tuberculosis. This fact is also supported by the negative animal experiment described. On the other hand, it is equally clear that the pathological process set up by it in the lungs bears no little resemblance to one set of changes at least met with in the same organs in tuberculous pneumonias. We notice in both cases the same tendency to infiltration of the framework of the lungs, degeneration of the inflammatory exudate, and to necrosis and solution of the tissues and exudate. In the particular case under consideration one point of difference is to be seen in the dearth of proliferated and desquamated alveolar epithelial cells within the exudate. But this difference is insufficient to characterize the two processes as essentially distinct. On the other hand, the impression is obtained that the solution of the exudate and framework of the lungs is going on more rapidly, from a greater number of centres, than is the case in ordinary cases of caseous pneumonia.

The analogy of the two processes is strengthened by the finding of tubercle-like nodules in the lungs amid the diffuse exudate. These were, it is true, few in number; but in structure they could not be distinguished from veritable tubercles, and they were proven to have been caused by the streptothrix under consideration, by the demonstration of the organism within them. It will be recalled that sections of the lungs stained for tubercle bacilli by the ordinary method failed to reveal the presence of this micro-organism.

When we come to consider the significance of the tubercles in the peritoneal cavity, it must be admitted that the case in favor of their

streptothrix origin is less clear and convincing. But I am inclined to the belief that they owe their presence to the same parasite, which, however, owing to differences in the location and function of the parts, appears under somewhat different morphological forms. In view of the great numbers of micro-organisms contained within these nodules, it seems justifiable to conclude that they would have been demonstrated by the usual stains for tubercle-bacilli, had they been of this nature. But I am unwilling to draw very wide-reaching deductions from this fact alone. The differences in morphology of the organism, when compared with the tubercle bacillus, may again be remembered, without, however, laying too much stress on small distinctions. Perhaps the most convincing argument is to be found in the existence of similar nodules in the lungs, whose etiology seems established. In these formations the organism appears under a simpler morphology than when growing freely within the lung substance.

It would not be profitable, nor would it conduce much to a clearer understanding of the variation in morphology, to dwell upon the influence of environment upon the growth and morphological character of the bacteria; but it should be borne in mind that our knowledge of such variation is constantly increasing and even now includes some of the commoner and more ordinary bacterial forms.

If in the comparison of the lesions in the lungs with those of caseous pneumonia, sufficient proof has been brought of their great similarity to these, more conclusive evidence is at hand of the essential identity of the nodules in the peritoneal cavity with the tubercles caused by the bacillus tuberculosis. It is believed that upon histological grounds alone no distinction could be made.

From these considerations there can be little doubt that the streptothrix described is the cause of the pathological process met with in the lungs and peritoneal cavity, and that in the latter the pathological picture of the disease resembles so nearly tuberculosis in human beings that the two diseases can be separated only by the demonstration of the causative micro-organism in each case. It is, of course, not without the pale of probabilities that at some future time a clinical picture differing from that of true tuberculosis may come to be established for this disease.

If this conception of the pathological changes produced by the streptothrix under consideration be true, the process must be admitted to be a form of pseudo-tuberculosis in man for which the name at the head of this paper is proposed.

The question now arises whether a similar pathogenic streptothrix, producing analogous lesions, has thus far been described. There seems little doubt from the descriptions given of the pathogenic species of this organism in the introductory pages of this paper, that the forms there mentioned differ from the one under consideration. At least no good grounds are at hand for concluding that in any case they are identical or even closely related. Not so, however, with the streptothrix described by Buchholz,* whose publication appeared simultaneously with the preliminary communication concerning the streptothrix pseudo-tuberculosa. It may perhaps be profitable to consider Buchholz's findings more in detail.

The patient was a steel worker, 38 years old, who became suddenly ill 5 weeks before admission to the Institute for Infectious Diseases in Berlin. He entered on November 19, at which time there was absolute dulness over the entire right side of the thorax, with rales and bronchial breathing. The sputum showed numerous streptococci but no tubercle bacilli. The fluid obtained by puncture from the pleural cavity contained streptococci. On November 22, 300 cc. of pus evacuated from the pleural cavity again showed streptococci. Death occurred on December 12th. The autopsy showed the right lung to be united throughout with the costal pleura; the left lung was bound to the diaphragm. The portion of lung corresponding with the adhesion to the diaphragm showed a consolidated focus the size of a hen's egg, which on section appeared to be necrotic. In the pleura covering the right lung were two formations which led directly into two large cavities, themselves communicating, filled with purulent contents and exhibiting ragged and uneven walls. The pus had a fetid odor; the remainder of the right lung, excepting the apex, was firmly consolidated. The rest of the organs showed no abnormality. The anatomical picture suggested an advanced tuberculosis, except that the edges of the cavities were more ragged. Tubercle bacilli could not be demonstrated; streptocci were, however, contained in the pus.

* Buchholz, Ueber menschenpathogene Streptothrix. Ein Beitrag zur Aetiologie des acuten Lungenzerfalls. *Zeitschr. f. Hygiene u. Infectionskrank.*, xxiv, 1897, 470.

The microscopical examination of sections was negative for tubercle bacilli; the histological picture, too, was regarded as showing differences from that of tuberculosis. The infiltrated parts of the lung presented more the appearance of an acute pneumonic exudate; but in places the interstitial infiltration was so marked as to obscure the structure and the cells were undergoing necrosis (fragmentation, etc.). Stained by Gram's method the firm, infiltrated and necrotic areas showed a rich network of delicately branching threads of about the thickness of tubercle bacilli, which never appeared in the form of long bacilli, but suggested "lines sketched by the trembling hand or the gnarled branches of the oak." Coccus-like and short bacillary forms were not found. The streptococci were contained in the portions of the lung which were consolidated, the alveolar walls being thickened and the alveoli in part atalectic. They were also present in the secretion of inflamed bronchi and in the interstitial lymph-spaces, from which situations the streptothrix was absent. The streptococci are regarded as having been secondary invaders. Cultures were not obtained from the lungs, the plates being overgrown with a variety of proteus. Animal experiments seem not to have been carried out. It is interesting to note that the same difficulty in staining the micro-organism was experienced as recorded in my case, and the most satisfactory preparations were obtained by the use of Gram's method, in which aniline-oil replaces the alcohol.

The resemblance of this case to the one described by me is very striking, but there are also points of marked difference. As regards the latter, the absence of all nodular formations suggesting tubercles and the extensive cavity formation in the right lung stand out in great contrast. On the other hand, it is conceivable that the cavities in Buchholz's case merely represent a later and more advanced stage of the condition present in mine. The description of the micro-organism is strikingly like that given for the streptothrix pseudo-tuberculosa, and in further support of their probable identity, I may mention that Dr. Rabinowitsch, who kindly carried slides of my case to Berlin with her, reports that Dr. Buchholz, after examining the preparations and comparing them with his own, regards them as probably identical.

At the last meeting of the Congress for Internal Medicine, held in Wiesbaden, Scheele and Petruschky* exhibited cultures and micro-

* Scheele and Petruschky, Culturen u. Präparate einer menschen-pathogenen Streptothrix-Art. Verhand. des XV. Cong. f. innere Medicin, 1897, 550.

scopical preparations of a pathogenic streptothrix obtained from a woman, aged 56 years, whose history, in brief, is as follows:

The patient gave a family history of tuberculosis, but had herself always been well. In November of the previous year she nursed a sister who died of tuberculosis. In February she suffered with what was supposed to be influenza; in March a cystitis developed, and about the middle of April several small tumors appeared in the skin about the joints (hip, sterno-clavicular), and later in the thigh, gluteal region, etc. The tumors suppurated. In the beginning of May the left upper lobe of the lung showed a fairly intense infiltration. The sputum was examined, but no tubercle bacilli could be found; on the other hand, masses of a "fungus" suggestive of penicillium were made out and a similar organism was demonstrated in the pus from the tumors. The patient died on June 6th. The protocol is not given in this preliminary report. The micro-organism is, however, described as having been obtained in pure culture from the pus from the tumors; in the lung it was admixed with the bacillus influenzæ. In both situations it was abundant. The parasite is said to consist of fine, branching threads without showing any evidences of fructification. The complete description is reserved for a later publication.

In this instance it would be merely a matter of conjecture to consider that the lesions in the lungs and the streptothrix are similar or identical with those described by me; indeed, the clinical course of the case is not especially suggestive of similarity. The important consideration for the present is not to attempt a classification of the several pathogenic streptothrix forms now known, but to recognize their importance in human pathology, and more especially that the lesions, which they produce in the lungs, may be suggestive of certain forms of pulmonary tuberculosis both in their clinical course and their anatomical appearance. Moreover, as the parasite is doubtless thrown off with the expectoration, it should be sought in doubtful cases in the sputa; and for this purpose the usual methods of staining for tubercle bacilli are not suitable. I should recommend the use of the modification of the Gram-Weigert stain with which the parasite was demonstrated in smear-preparations and in sections.

I wish to thank Dr. W. M. Gray for the photomicrographs which accompany this paper.

DESCRIPTION OF PLATE XLI.

Fig. 1. Photomicrograph of smear-preparation from lung.

Fig. 2. Photomicrograph from section of the lung. Both show the streptothrix: Fig. 1, free; Fig. 2, in intimate association with the exudation. Photographs by Dr. Gray, of Washington.

Fig. 3. Section of lung stained in hæmatoxylin and carbol-fuchsin. Zeiss 1/12 in homog. immersion objective. The characteristic beading of the streptothrix is well shown.

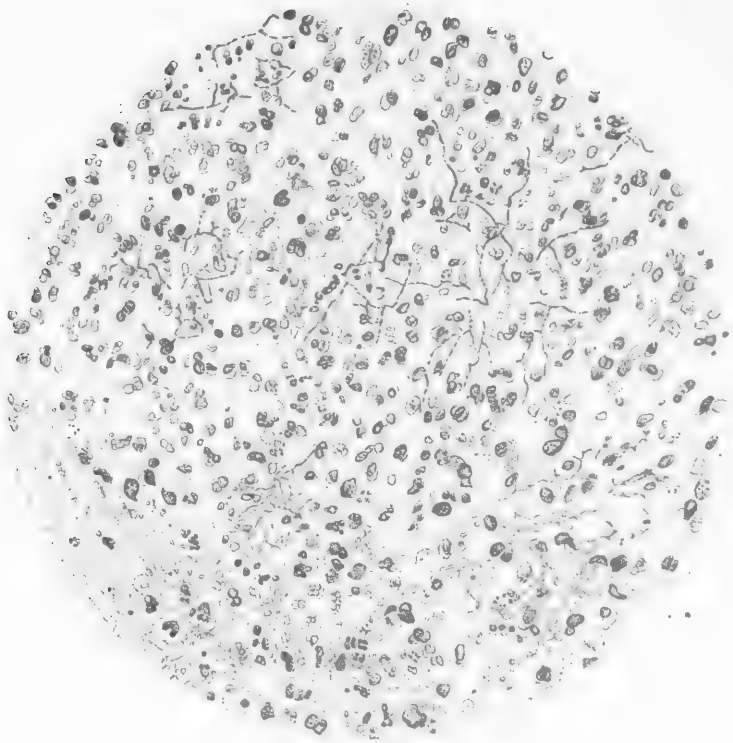

FIG. 3.

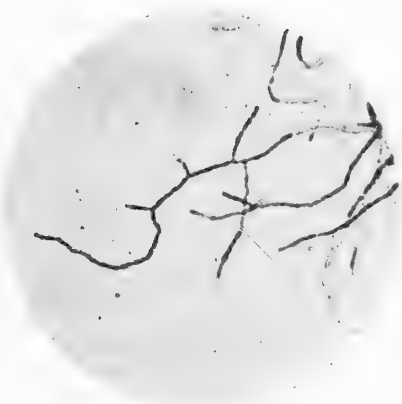

FIG. 1.

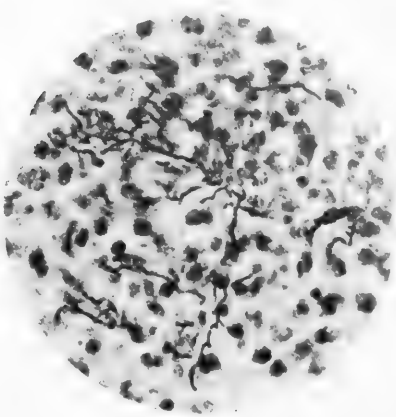

FIG. 2.

A COMPARATIVE STUDY OF BOVINE TUBERCLE BACILLI AND OF HUMAN BACILLI FROM SPUTUM.

By THEOBALD SMITH, M. D.

(From the Laboratories of Comparative Pathology, Harvard Medical School, and the Massachusetts State Board of Health.)

The absolute identity of tubercle bacilli infecting mammalia has been so generally assumed and the assumption used as a basis for the enactment of sanitary measures having for their object the prevention of any transmission of tubercle bacilli from animals to man, that any one who would attempt to question this identity must be prepared to meet considerable scepticism. Taking a broad biological position, we have every reason to examine into the assumed identity of the bovine and the human bacillus, because both the human and the bovine species are victims of a tuberculosis, presumably transmitted in most cases from one individual to another of the same species, and because the adaptation of a highly parasitic organism to one of these species for centuries may possibly deprive it of much of its power to multiply in the other. Or the reverse may be true. The adaptation of the bovine bacillus to a larger, more vigorous organization may thereby render it more dangerous to man. Assumptions of this sort stimulate inquiry but do not furnish us with positive information. This can be gained only by most tedious investigation.

In 1896 I presented to the Association of American Physicians* a comparative study of a bovine bacillus and a presumably human bacillus which had passed from a tuberculous subject to an animal pet (*Nasua narica*, 'Coati'). The differences which appeared during this work were so clear, both as regards morphological and biological characters, that a further study was indicated. Imbued with the idea that in the minor, though constant, differences of closely related or apparently identical pathogenic bacteria many unexplained phenomena may

* *Trans. Assoc. Amer. Physicians*, xi (1896), 75.

have their source, and that a comparative study of tubercle bacilli under as nearly the same conditions as can be maintained may lead to results of value to human pathology, I took up the subject anew. A consultation of the literature treating of the character of tubercle bacilli had impressed me with the carelessness shown by most writers subsequent to Koch, concerning the source of the cultures used by them. Papers written on methods of cultivating the presumably human bacillus were really devoted to the avian bacillus. Fischel,* though drawing far-reaching conclusions on the relation of human to avian bacilli, makes no statement concerning his cultures save that they were "mammalian."

There being, therefore, no information to be gathered from former publications, the task that presented itself was to study a number of human and bovine tubercle bacilli, and, if possible, tubercle bacilli from other mammalia, side by side, in order to determine whether constant differences, of whatever nature, could be demonstrated. Such differential characters might then be applied in studying different forms of human tuberculosis, especially those cases which are supposed to have been derived from animals, more particularly from cow's milk.

The delay inseparable from the study of this disease has not permitted me to examine more than a limited number of cultures. Including the two described in 1896, 7 sputum and 6 bovine cultures have been isolated and studied; also one animal culture presumably derived from sputum, one culture each from the horse, the cat and the pig.

In Table I are given brief accounts of the subjects from which they were obtained. For the notes on the cases of phthisis I am indebted to a number of physicians who have very generously given their time in tracing the subsequent history.

* *Fortschr. d. Med.*, x (1892), 908. See also my former article for further references.

TABLE I.

Designation of culture.	Beginning of artificial cultivation.	Source of culture.	History of Case.
Sputum I	July 20, 1896	Peabody, Mass.	Sputum discharged, June 24, 1896, by a young Irish woman. Apex and upper lobe of left lung involved. Slight fever. Sputum contains a considerable number of bacilli. Recovered and worked during summer. Good health in the fall of 1896, with gain in weight. Feb., 1898, in good health, works daily. No cough, expectoration or other symptoms of illness. Over the formerly involved region "the respiratory murmur much diminished down to 3rd rib. Increased vocal resonance, increased fremitus and dullness on percussion." Patient has moved from place to place.
Sputum II	Nov. 21, 1896	New Bedford, Mass.	Sputum containing many tubercle bacilli, discharged Oct., 1896, by a young man confined in prison about one year. Earliest appearance of symptoms not accurately known. Slight cough noticed in Aug., 1896, but no definite physical signs at that time. Grew steadily worse, so that in November he was in bed, weak and emaciated and also suffering with diarrhœa. He died in March, 1897.
Sputum III	Feb. 15, 1897	Norwood, Mass.	Sputum discharged, Jan. 12, 1897, by a man aged 45 years. Duration of disease about six months. Laryngeal tuberculosis chiefly. Difficulty in swallowing and huskiness of voice, which has been gradually increasing. Died Feb., 1897. The physician in charge had drawn off about a litre of serum from thorax 4 or more years ago. Well thereafter until fall of 1896. Apex of lungs slightly involved towards end of disease. Patient has traveled much in this country.
Sputum IV	Feb. 16, 1897	Melrose, Mass.	Sputum containing but relatively few bacilli, discharged Jan. 7, 1897, by a female servant, 22 years old. Physician in charge saw her first Dec., 1896. She complained of hoarseness and cough which began about 6 months ago. Signs of anæmia and cessation of menstrual flow. Placed in hospital, where she remained 8 weeks. At end of April health quite re-established. A subsequent report mentions her visit to Canada after her recovery and her return to Massa-

TABLE I.—*Continued.*

Designation of culture.	Beginning of artificial cultivation.	Source of culture.	History of Case.
			chusetts. She is now (Jan., 1898) at service in another town. She has a troublesome cough and is under the care of a physician.
Sputum V	July 7, 1897	New Hampshire.	Sputum discharged, June, 1897, by a man 25 years old. Had been troubled with a cough for about 2 years, and apices reported involved. Patient feels well in the country, but cannot carry on his trade (printer) in the city. In Jan., 1898, he was reported to be much improved in every way.
Sputum VI	July 14, 1897	Winthrop, Mass.	Sputum discharged, June 16, 1897, by a woman 20 years old. Tubercle bacilli numerous. History not obtainable. Patient died about 6 months later.
Sputum VII	Jan. 18, 1898	Haverhill, Mass.	Sputum containing many bacilli, discharged by an Italian laborer, Dec. 15, 1897. Age of patient 27 years. Returned to Italy in Jan., 1898.
Nasua narica (Coati) human sputum (?)	July 26, 1894	Washington, D. C.	Autopsy, May 12, 1894, revealed primary intestinal infection, as mesenteric glands were farthest advanced in the disease. Omentum studded with tubercles. Extensive embolic tuberculosis of lungs. Household pet of tuberculous man. At Washington Zoological Park, where animal was kept after death of owner, other animals of same species remained well.
Bovine I	Dec. 1, 1894	Virginia.	Bull with advanced generalized disease. Great enlargement of lymph glands of head and thorax. Tuberculosis of the bones. Periodic tympanites due to enlarged mediastinal glands. Killed.
Bovine II	Nov. 28, 1896	Lawrence, Mass.	Cow having multiple necrotic foci in large caudal mediastinal gland. Foci dry cheesy; process evidently old. In one lobe of lung a focus 1½ inches in diameter with centre softened. In udder small nodules containing actinomyces. Killed.
Bovine III	April 19, 1897	Carlisle (?), Mass.	Cow killed, March 19, 1897, at Brighton (Boston) abattoir. Quite extensive disease of lungs and liver.

TABLE I.—*Continued.*

Designation of culture.	Beginning of artificial cultivation.	Source of culture.	History of Case.
Bovine IV	Apr. 23, 1897	Billerica, Mass.	Cow killed, March 19, 1897, at Brighton abattoir. Disease restricted to one of dorsal mediastinal glands. The gland is moderately enlarged and contains a number of well-defined tubercles, 3 to 4 mm. in diameter, dry cheesy throughout, and easily peeled out of gland tissue. No surrounding infiltration. Evidently a mild stationary case.
Bovine V	Apr. 23, 1897	Carlisle, Mass.	Cow killed, March 19, 1897, at Brighton abattoir. Disease has produced a focus in the lungs, and involves all dorsal mediastinal glands, which are considerably enlarged. One of the portal glands is enlarged, and permeated with a network of necrotic lines.
Bovine VI	Dec. 24, 1897	Newton, Mass.	Caseous matter from a focus in the lungs of a cow 2 to 3 years old. The focus several inches in diameter, probably not more than 3 to 3½ months old, since the cow gave no tuberculin reaction 4 months ago. Dorsal mediastinal gland involved.
Swine I	May 8, 1896	Southboro, Mass.	Lungs and one gland (presumably from mesentery) received at laboratory, March 18, 1896. The herd lived under a cow stable, and the infection was probably bovine in origin. Lungs studded with firm, centrally cheesy or calcified nodules from 1 to 4 mm. in diameter. The lymph gland contained many foci with cheesy centres.
Cat I	Mar. 9, 1897		Case reported by L. Frothingham, V. M. D., in the Journal of the Boston Society of Medical Sciences, March, 1897. Tuberculosis of a mesenteric gland with miliary tuberculosis of lungs, liver and spleen. Tubercle bacilli in capillaries of lungs and liver. Guinea-pig inoculated by Dr. Frothingham, and kindly transferred to me.
Horse I	Feb. 20, 1897		Lungs of a horse from Brighton Rendering Establishment. Guinea-pig inoculated from tubercles by Dr. L. Frothingham. History of animal not known.

It will be noticed that these cultures come from a variety of cases, both mild and severe. Of the patients yielding the sputum bacilli, three are dead, two still sick, one has apparently recovered, and one, a recent case, is now not accessible. Of the bovine cases two were slight, two advanced and two of moderate intensity. Of the other mammals nothing very definite is known.

The bacilli were isolated in all cases by inoculating guinea-pigs with tuberculous tissue or sputum containing bacilli. With sputum the injection of a suspension in bouillon into the abdominal cavity is the most satisfactory. Subcutaneous inoculation may induce a very chronic disease with older (500-gramme) guinea-pigs, which is not so likely to lead to successful cultures because of the scarcity of bacilli in the tuberculosis foci. In nearly all cases the guinea-pigs were chloroformed after three or four weeks, to prevent secondary infections occurring in the final stages of the disease.

Inasmuch as a more general study of tubercle bacilli is desirable, I shall go somewhat into detail concerning the methods I have employed in making cultures. Having had practically nothing but failures until I adopted the procedure to be described, I can recommend it without reserve. There is nothing new about it, excepting in the combination of details. Of 18 attempts to isolate the bacillus, 17 were successful. The one failure was probably due to the slow progress of the inoculation disease in the guinea-pig.

Throughout the work dog's serum was used. The dog was bled under chloroform and the blood drawn from a femoral artery, under aseptic conditions, through sterile tubes directly into sterile flasks. The serum was drawn from the clot with sterile pipettes and either distributed at once into tubes or else stored with 0.25 to 0.3 per cent chloroform added. Discontinuous sterilization was rendered unnecessary. The temperature required to produce a sufficiently firm and yet not too hard and dry serum is for the dog 75° to 76° C. For horse serum it is from 4° to 5° lower. The serum was set in a thermostat into which a large dish of water was always placed to forestall any abstraction of moisture from the serum. About three hours suffice for the coagulation. When serum containing chloroform is to be coagulated, I am in the habit of placing the tubes for an hour or longer in a water-bath at 55°-60° C., or under the receiver of an air-pump, to drive off the antiseptic. This procedure

dispenses with all sterilization excepting that going on during the coagulation of the serum. It prevents the gradual formation of membranes of salts, which, remaining on the surface during coagulation, form a film unsuited for bacteria. Tubes of coagulated serum should be kept in a cold closed space where the opportunities for evaporation are slight. They should always be kept inclined.

The ordinary cotton-plugged test tubes I do not use, because of the rapid drying out permitted by them, as well as the opportunities for infection with fungi. Instead, a tube is used which has a ground glass cap fitted over it. This cap contracts into a narrow tube plugged with glass wool. This plug is not disturbed. The tube is cleaned, filled and inoculated by removing the cap. With sufficient opportunity for the interchange of air very little evaporation takes place, and contamination of the culture is a very rare occurrence. In inoculating these tubes, bits of tissue, which include tuberculous foci, especially the most recent, are torn from the organs and transferred to the serum. Very little crushing, if any, is desirable or necessary. I think many failures are due to the often futile attempts to break up firm tubercles. Nor should the bits of tissue be rubbed into the surface, as is sometimes recommended. After a stay of several weeks in the thermostat I usually remove the tubes and stir about the bits of tissue. This frequently is the occasion for a prompt appearance of growth within a week, as it seems to put certain still microscopic colonies in or around the tissue into better condition for further development. The thermostat should be fairly constant, as urged by Koch in his classic monograph, but I look upon moisture as of more importance. If possible, a thermostat should be used which is opened only occasionally. Into this a large dish of water is placed, which keeps the space saturated. Ventilation should be restricted to a minimum. As a consequence, moulds grow luxuriantly and even the gummed labels must be replaced by pieces of stiff manila paper fastened to the tube with a rubber band. By keeping the tubes inclined, no undue amount of condensation water can collect in the bottom, and the upper portion of the serum remains moist. The only precaution to be applied to prevent infection with moulds is to thoroughly flame the joint between tube and cap as well as the plugged end, before opening the tube. When test tubes are employed it is well to dip the lower end of the plug into sterile molten paraffine and to cover the tube with a sterilized paper cap. The white bottle caps of the druggists are very serviceable.

In pursuing this method of obtaining cultures, the question arises: May not attenuated or more saprophytic forms exist in the sputum

besides those more pathogenic, and may not the former get into our cultures rather than the latter? This question cannot be answered with entire satisfaction. If we assume, for the sake of illustration, that the original disease was due to perhaps one inhaled bacillus, the progeny of this bacillus may be reinforced by others inhaled which have more saprophytic and less pathogenic tendencies, and which multiply in the cavities and in the sputum to the partial suppression of the original form. If we make cultures directly from sputum this hypothetical danger could not be evaded. The inoculation of the guinea-pig and the three or more weeks elapsing before cultures are made is to a certain degree a safeguard, as it gives the more pathogenic form an opportunity to assert itself by prompt multiplication. I am not inclined to look upon this danger of substitution as a real one. At the same time, it may be best always to choose those successful cultures from the guinea-pig which have come from metastatic rather than local (peritoneal) tubercles. In my work I obtained in most cases cultures simultaneously from 3 or 4 organs, and any differences in these original cultures were not manifest. Only one was used in the comparative studies. Of 7 sputum cultures, 3 were derived from the omentum and 4 from the spleen. Of one the source is not noted. For the bovine cultures no such doubts can be raised, because the foci are closed and not accessible to more saprophytic types.

In the study of the various cultures the conditions were maintained as nearly uniform as possible by using tubes of serum prepared at the same time, and keeping the inoculated cultures in the thermostat in the same trays. For the microscopic examination a little of the surface growth was rubbed between two cover glasses, allowed to dry in the air, and then the whole series to be compared were fixed all together in the hot-air sterilizer at 120° C. for 20 to 30 minutes. For staining, carbol-fuchsin was used cold, and the decolorization effected with a 10 per cent aqueous solution of sulphuric acid acting for from 15 to 30 seconds. Cover-slips were also decolorized with $\frac{1}{2}$ per cent acetic acid, some simply washed in water, others were stained according to Gram-Weigert. In all cases the members of the series studied were treated alike and at the same time, thus eliminating minor, often unavoidable, variations. In all cases the characters to be described refer to dog-serum cultures. These were renewed every 3 to 6 weeks, as many at a time as was possible. The regularity of re-inoculations was now and then interrupted by a beginning infection of the tube with moulds, or a too feeble growth, necessitating

earlier transfer to fresh tubes. As a rule the cultures were kept continuously in the thermostat until the next transfer was made.*

The tendency of most species of bacteria to vary slightly from tube to tube, either in size, form, or staining capacity, is also characteristic of tubercle bacilli, so that repeated examination of series of cultures is necessary to obtain what might be called a composite photograph of their most permanent characters or peculiarities. The statements made below as to the morphology of the bacilli under observation are based on the repeated examination of hundreds of slides of which the conditions of growth of the bacilli, the coagulating point of the serum, and the staining manipulations had been carefully noted. The cultivation of tubercle bacilli requires, even under the best circumstances, constant personal attention, and those not prepared to give it had better not attempt it seriously. Success is obtainable along lines other than those suggested by me. Koch succeeded by using beef's serum in ordinary plugged test tubes. He is, however, the only investigator, I believe, who has studied series of cultures of tubercle bacilli.

MORPHOLOGICAL AND BIOLOGICAL CHARACTERS.

With one exception to be noted below (Sputum I), the human bacilli grew from the start much more vigorously than the bovine bacilli. With several the rapidity of growth was surprising. After two weeks these cultures appear as a whitish surface layer of a pearly lustre, of varying thickness. The bovine cultures show merely discrete colonies, or a thin, uniform layer having the appearance of ground glass.† This difference in the vigor of growth has, in general, maintained itself, with the slowly increasing tendency of all cultures to multiply more rapidly.

The size of bovine bacilli in the various cultures was quite constant. They were all quite short, usually about 1 to 1.5 μ long, more rarely 1

* It is always desirable as a precautionary measure to retain 3 or 4 older transfers of any culture in a cool, dark place, so that it may not be lost through some accident or contamination. Now and then culture-media will prove unsatisfactory. In such cases, the earlier tubes must again be resorted to. I have noticed that upon all the serum tubes from one dog, growth was feeble. The liver and the kidneys of this animal showed considerable fatty changes, although the animal had not been ill. Possibly overfeeding may be the cause of the difficulty.

† In some tubes a peculiar coppery discoloration may extend over the entire surface growth. Cultures from these are fertile, however. There is nothing in the microscopic appearance of the bacilli to explain this change. It is not an uncommon feature of cultures of feeble vitality, or of unsatisfactory culture-media.

to 2 μ. These measurements do not tend to change appreciably with prolonged cultivation. The bacilli are straight, not very regular in outline. Some are broader at one end than at the other, some broader in the middle than at either end, *i. e.* spindle-shaped. Some may be so short as to resemble oval cocci. With the human bacilli the form was not so constant. The earliest cultures of Sput. II, IV, V and VI contained forms from 1 to 2 μ long, hence closely approximating the bovine forms. Others (Sputum III) may be longer from the start. In all, however, there is a tendency, not noticed among bovine cultures, to grow longer under artificial cultivation. Perhaps the greatest contrast in form, which has remained so for three years, is illustrated by the bovine and the Nasua (human) culture described in 1896. In the latter the bacilli are from 2 to 3 times the length of the former.

In the earliest cultures, therefore, morphological differences are not necessarily characteristic, and cannot aid us in attempts at determining the origin of cultures. Cultures containing only small forms have been noted by Metchnikoff and Straus. The latter states that even the avian variety may assume very small dimensions under certain circumstances.

When the serum to be used for cultures is coagulated at a temperature of 71° to 72° C., instead of 75° to 76° C., certain modifications are manifest. All bacilli which have developed on a serum having a firm surface, and which is obtained at the higher temperature, absorb the dye promptly and become brilliantly stained. When a softer (71° C.) serum is employed, whose surface is easily depressed, torn and broken by the platinum needle, the culture, which may have become either more or less vigorous than before, now contains a large number (from 50 to 90 per cent) of bacilli, which stain quite feebly. Uniformly dispersed among them are deeply stained forms. This feeble stain is noticed when bacilli are not treated with decolorizing agents at all, and the appearance of the cover-slip preparation is not materially altered by the acid.*

While both human and bovine bacilli are affected by the softer serum, the former show a much greater degree of change. The bacilli are very feebly stained and show outlines often very indistinct. Further than

*** Since the completion of the manuscript further studies of this phenomenon have convinced me that at least some of the feeble staining is due to the formation of a capsular substance, which interferes with the penetration of the dye. This substance, which is produced in greatest abundance by the human cultures, is not infrequently detected as an irregular, unstained envelope around a single bacillus or a group. It is not stained by the procedure recommended by Prof. Welch for staining capsules.**

this, the human bacilli, on the softer serum, tend to become longer and slightly curved. In some cultures this change is very pronounced, in others less so. With the bovine forms there is no elongation, although the bacilli may be less brilliantly stained than on firm serum. Of seven human cultures studied, all have exhibited this tendency to become slender, while none of the bovine bacilli have been seen to change their size to any appreciable degree. Some of the bovine cultures from softer serum did not even show any feebly stained bacilli.

Another modification in form which manifests itself upon the firmer serum is the appearance of round, deeply stained bodies, at or near the end of the usually slender bacilli, very rarely in the middle. These bodies, from 1.5 to 2 times the diameter of the rod, take a brilliant stain in carbol-fuchsin, contrasting with the more feebly stained, less compact rod in which they are imbedded. These bodies, which have a striking resemblance to spores, may be present in all the rods of any one preparation, or in a certain proportion only. They have been seen in Nasua, Sputum III, IV and VI. In the bovine cultures bodies of this nature are occasionally recognizable, but owing to the shortness of the bacilli they do not stand out distinctly. Of much more frequent occurrence in these cultures, often the only forms present, are short fusiform or spindle-shaped rods, the central swelling being also deeply stained, and perhaps corresponding to the terminal body of the human forms. These bodies have been seen by Ehrlich, Nocard and Roux, Metchnikoff, Cornil and Babes, Czaplewski and Straus. They have been variously interpreted as spores, and bacilli containing them have been successfully double-stained. Their significance remains unsolved. The most probable explanation is a degeneration or involution form. They may, however, represent some resting stage and correspond to the short, brilliantly stained bodies frequently seen in giant cells, and not recognizable as bacilli. In unstained films, mounted in water, they cannot be distinguished even with a very narrow pencil of light. An increased refrangibility cannot be made out.

Experiments to determine the length of time required at 60° C. to destroy both human and bovine forms have thus far not revealed any differences. The tests are reserved for a special publication.

A study of the relative resistance of the bacilli to drying has not yet been attempted.

Among the sputum cultures is one (No. 1) which grew so feebly on dog's serum that its cultivation was finally given up. The history of the case is briefly given in Table I. Since the comparative tests of the pathogenesis of this culture are very incomplete, I shall give briefly the

results of the inoculation of the sputum as evidence that the organism was in reality a tubercle bacillus.

June 27, 1896. Guinea-pig, weight 640 grammes, receives into abdomen 0.5 cc. of bouillon in which some sputum is suspended.

July 20. Weight 457 grammes. Animal rather dull, with coat slightly staring. Very little resistance to handling. Chloroformed.

A few subserous tubercles around point of injection. Omentum represented by a cylindrical mass of neoplastic tissue, about 1 to 2 cm. in diameter, firm, speckled with whitish dots indicative of necrosis. Tubercles on diaphragm and testicles. Liver spotted with small, irregular, not well-defined, yellowish necroses. Spleen slightly enlarged, with Malpighian bodies prominent. Bronchial and sternal glands enlarged, with central necrosis. Lungs free from visible tubercles.

The pathogenic power of this sputum seems to have been equal to that of most sputa examined. One guinea-pig of 672 grammes, inoculated precisely like the preceding, died 46 days after inoculation, with more pronounced lesions of the liver and spleen. A third guinea-pig of 370 grammes, which had received the same quantity subcutaneously, weighed a trifle more on the 49th day (390 grammes) than at the beginning. On this day it was chloroformed and examined. The typical ulcer with surrounding tubercles in the subcutis and a few tubercles in the omentum were seen. The liver was pale and fatty, the spleen enlarged to several times the normal size and uniformly permeated with grayish (one mm.) foci, but without necroses. The lungs were studded with small, partly necrotic tubercles. The bronchial and sternal glands were large, with necrotic centres.

The first culture showed somewhat slender but well-stained bacilli. In tubes subsequently inoculated, the bacilli multiplied almost exclusively in the condensation water, which became milky in appearance. In this the bacilli appeared in roundish clumps among cholesterin crystals. The bacilli were imbedded in a colorless material which formed the cohesive clump. The stained bacilli presented no unusual characters save in one culture (after about six months of total cultivation), in which fully one-half of all the quite slender bacilli appeared as series of transverse, apparently unconnected stained blocks, simulating streptococci. It is unfortunate that more animal tests were not made with this stock. As I was expecting a more vigorous culture from month to month, tests were postponed until conditions should approximate those of the other cultures. These were, however, not realized. The only test upon a rabbit is given in Table III., The cultivation was finally abandoned.

Of the other animal bacilli the cultures from swine were identical with the bovine cultures in morphological and other characters. Those of the horse and the cat were also either identical with the bovine bacilli or they very closely resembled them.

If we undertake to summarize the observations made with microscope and culture tube upon these bacilli, we somewhat hesitatingly formulate the following general statements:

1. Bovine and other animal bacilli (except *Nasua*, which is regarded as coming from man) grew less vigorously for a number of generations than the sputum bacilli. Sputum I is an exception and is probably an atypical form.

2. Bovine bacilli are much less influenced by certain modifications of the culture-medium.

3. Bovine bacilli tend to remain short; human bacilli are either more slender from the start or become so during cultivation.

PATHOGENESIS.

The most important means of demonstrating identity or divergence of characters of pathogenic bacteria is the test upon animals. This test is a purely physiological one, but in the application of experimental results from the domain of bacteriology to medicine, morphological characters must give way to physiological.

That there might be differences among tubercle bacilli from different species of mammals presented itself as a possibility to Koch and is referred to in his monograph. He, however, failed to recognize any differences worth mentioning. There are several reasons for this. In the first place, Koch was at that time endeavoring to prove to the world that the tubercle bacillus is the cause of tuberculosis. Minor details received no attention because of the great and difficult task immediately before him. In the second place, the course of tuberculosis is essentially a function of the number of bacilli introduced. Variations in the dose result in corresponding variations in the length of the disease, in its final termination, and in the extent and distribution of the lesions. This fact Koch himself recognized in discussing the results following the intra-ocular inoculation of varying quantities of culture material in rabbits. He did not, however, attempt to adjust

carefully the quantity to be injected or to determine the effect of varying quantities of bacilli upon any of the species used by him. Curiously enough, no one following him seems to have considered it worth while to extend his work by more accurate comparative tests. That such work is likely to be fruitful the following experiments amply demonstrate.

Guinea-pigs. The inoculation of this species with tubercle bacilli from the various cultures brought out few well-defined differences. The great susceptibility of guinea-pigs to tubercle bacilli is responsible for the general acceptance of a common level of pathogenic power for all mammalian tubercle bacilli. This tendency to unify physiological characters because of a more or less uniform action upon very susceptible species has led to frequent failure on the part of experimenters to recognize important variations among species of bacteria, variations probably having much influence upon the course of the disease contracted in the natural way. There are, however, certain differences in the action of bovine and sputum bacilli upon guinea-pigs which enable us to recognize even in these very susceptible animals the much greater virulence of bovine bacilli. It is probable that by a graded dosage of tubercle bacilli more marked differences might be brought out than I am in position to present.

It has already been stated that all the cultures in hand had been obtained by inoculating the tuberculous tissue or the sputum into guinea-pigs and isolating the bacilli from them. To obtain the bovine cultures, a small bit of tissue was placed in a subcutaneous pocket in the flank. For sputum bacilli the sputum was shaken up in sterile bouillon and the dilution injected into the abdominal cavity. In these preliminary experiments differences presented themselves between the two kinds of material. Although the sputum, as a rule, contained very many tubercle bacilli and the bovine tissue very few, yet the subcutaneous injection of sputum led to such a chronic disease, with comparatively slight lesions and a tendency to reparative changes in the liver, that the animals were very unpromising subjects for culture when chloroformed.* Hence the intra-abdominal inoculation in most cases. On the other hand, the disease due to bovine tissue placed in the subcutis never failed

* These statements apply only to guinea-pigs weighing 400 grammes and above.

to produce extensive lesions, with indications of certain death in 4 to 6 weeks, which was anticipated by chloroform when cultures were to be made. Leaving this preliminary series of inoculations, we will pass to those made with definite doses of tubercle bacilli in suspension.

The suspension was made by thoroughly rubbing the growth from blood serum upon the inside of dry sterile test tubes with a heavy spatula-like platinum wire. This procedure results in the breaking up of clumps and the coating of the tube with masses of bacilli. Bouillon was then poured in and the thoroughly stirred suspension allowed to stand for several hours until all coarser particles had subsided. The resulting suspension was diluted, if necessary, until its density was nearly equivalent to that of a bouillon culture of typhoid bacilli 20 to 24 hours old, and nearly as homogeneous. More accurate dosage might be obtainable by weighing the moist growth before suspending it in bouillon; but inasmuch as the weighed quantity may not all be brought into a finely divided state—a matter of importance in the intravenous injection of rabbits—the method adopted is probably less troublesome and equally accurate.

Of this suspension the usual dose, both for subcutaneous and intra-abdominal inoculation, was 0.5 cc. An examination of the dates in the various tables will show that many of the inoculations upon rabbits, guinea-pigs and cattle were made with the same suspension on the same day.

The guinea-pigs employed were all rather large, weighing, with few exceptions, from 400 to 700 grms. They had all been used in diphtheria antitoxin tests one to three months before. The rapid increase in weight was evidence of no disturbance of health at the time of inoculation. It is of importance to bear in mind that the animals were large, because the progress of tuberculosis differs essentially in growing and adult guinea-pigs, the latter manifesting a far greater resistance than the former, a resistance expressed by well-marked reparative or cirrhotic changes in the liver. The differences to be pointed out in the bovine and sputum series may possibly be nearly wiped out by the use of guinea-pigs weighing only 250 to 300 grms.

The disease induced by the intra-abdominal injection was so rapid in its progress for both bovine and sputum cultures that the virulence of both races appears nearly the same.

The shortest period between inoculation and death, when 0.5 cc. of the suspension was injected, is 7 days, the longest 21 days, with one

exception, that of Sputum VI, which was 38 days. In other cases the period was prolonged because of smaller doses. For Bovine I (0.2 cc.) it was 33 days; for Nasua (0.3 cc.) 41 days. If we take the largest series in which the injections were made at the same time and in which the conditions were identical—the series comprising cultures of Sput. IV, V, VI and of Bovine III, IV, V—we find the periods elapsing between inoculation and death to be respectively 21, 11, 38 for the sputum and 7, 9 and 10 days for the bovine cultures.

The disease due to the intra-abdominal injection of tubercle bacilli in the quantity used is peculiar in this, that there is always found after death a large quantity of an opalescent fluid in both pleural sacs. This fluid greatly compresses the lungs and is probably the immediate cause of death. When the disease is prolonged beyond 3 weeks, the pleural effusion is absent. A careful histological study of the various organs in this most acute form of the inoculation disease, which will probably furnish the explanation of the effusion, is still to be made. In the abdominal cavity there is, as might be expected, a general inflammation of the peritoneum, associated with some fibrinous exudation and usually a little viscid fluid, when the disease does not last quite ten days. Any prolongation beyond this brings a dense eruption of tubercles into view. The omentum is in all cases represented by a cylindrical mass of fused tubercles.

If we turn to the subcutaneous inoculations, the difference in pathogenic power is more clearly brought out.

In the same series just quoted, of the three sputum guinea-pigs, one died 44 days after inoculation, the others were chloroformed respectively 77 and 100 days after inoculation; the first having lost but little in weight, the second being a trifle heavier, but having extensive pulmonary disease. The bovine guinea-pigs died in 51, 41 and 40 days respectively. The sputum guinea-pig, which succumbed in 44 days, weighed at the start 130 grms. less than the lightest bovine guinea-pig. The lesions were slight, the lymph glands only moderately enlarged, the foci in the lungs not visible to the naked eye. The spleen was not enlarged. The liver was mottled with very many grayish specks. The microscopic examination showed lesions but slightly advanced and reparative changes with proliferation of bile-ducts in progress. In general, the tuberculous lesions in this animal did not warrant so early a death.

The histological examination of the tissues of the infected guinea-pigs was restricted to the lungs and the liver of those inoculated simultane-

ously under the skin with cultures of Sputum IV, V and VI,* and of Bovine III and V. These organs were chosen because of their peculiar behavior towards the infection. The liver is invaded first, and under favorable conditions cirrhotic changes may begin which in some cases apparently lead to an elimination of the disease, with considerable shrinking of the organ and furrowing of the surface. In the lungs the disease begins later, and if the animal survives the first 6 weeks, may grow steadily until most of the organ is involved.

Pieces of tissue hardened in Zenker's fluid or alcohol were cut embedded in paraffine. The sections, fastened to the slide with Mayer's albumen and water combined, were stained with hæmalum and eosin or picro-acid-fuchsin, or with eosin and methylene-blue. In some cases Weigert's fibrin stain was resorted to. All were stained for tubercle bacilli in carbol-fuchsin, differentiated in 1 per cent acetic or decolorized in 4 per cent sulphuric acid, and stained subsequently in Löffler's methylene-blue. The stain for tubercle bacilli was successful in the tissues hardened in Zenker's fluid. Even in the sections stained according to Gram-Weigert, the bacilli appeared distinctly, but in a peculiar form, often simulating short streptococci with the individual cells rather well separated, or series of short blocks with spaces between them. Here the stain fastens itself only upon certain segments of the bacillus, leaving the connecting elements entirely unstained. The part selected evidently stands in no relation to the bulging, deeply stained, spore-like bodies referred to above as occurring in cultures.

In discussing the histological changes induced by tubercle bacilli in the various animals inoculated, it has been my determination to steer clear of all controversial questions relating to the histogenesis of the tubercle or of the elements composing it, as being foreign to the scope of this etiological study. Only those features will be very briefly referred to which show pronounced variations and which serve to point out differences in the action of the bacilli.

In the liver of the inoculated guinea-pig, the proliferative lesions present in all cases examined consist of aggregations of the characteristic epithelioid cells, among which, here and there, scattering nuclei appear richer in chromatin, and which for convenience are designated lymphoid elements. The lesions, situated chiefly in the interlobular tissue, are surrounded with a wide or narrow zone of proliferating bile-ducts, which appear as single or forked lines or in the form of meshes. There is noth-

* Spleen in place of liver in this case, the latter having been rejected by mistake.

ing in the microscopic appearance of the liver tubercles to serve as distinguishing marks, excepting the much more extensive involvement of the liver tissue and the much greater abundance of tubercle bacilli in the bovine cases. In the latter there are added to these lesions those of another character, the simple necrosis of cell territories ushered in by a fading out of the nuclei and a deep stain with eosin. These areas probably represent the yellow cheesy areas so common, and often very large, in young tuberculous guinea-pigs. They are readily distinguishable with the naked eye from the grayish proliferative changes going on in the interlobular tissue. In the sputum cases the very rare areas of this character seen were very small.

In the lungs, the lesions are as a rule perivascular and peribronchial. There are no essentially distinctive features here, excepting the very great abundance of tubercle bacilli in the bovine cases. In Bov. III the bacilli are also found in large numbers in cell detritus occupying alveoli and small bronchi. In Sputum VI many alveoli in the periphery of the foci are nearly filled out with multinucleated cells resembling giant cells closely, but their contents are only pigment particles. In Bovine V similar cells are present, though in small numbers. These, as well as other cells in the alveoli, each contain several bacilli.

The action of tubercle bacilli from the pig, the horse and the cat upon guinea-pigs was nearly the same as that of the bovine cultures, and needs no special discussion here.

With this hasty, imperfect sketch we may conclude the notes on the tuberculosis of guinea-pigs induced with cultures, by simply adding that the bovine bacilli studied are more virulent than human bacilli, although, owing to the great susceptibility of this species, the differences are not likely to impress us unless we give much attention to experimental details. These differences may be summed up as consisting in the more rapid death of all guinea-pigs inoculated with bovine bacilli, the greater extent of the lesions and the far greater abundance of tubercle bacilli in them.

In Table II will be found, in part, the basis for these conclusions.

TABLE II.—GUINEA-PIGS.

Culture.	Total age of artificial culture.	Number of transfer.	Date of inoculation.	Age of culture used, in days.	Dose in cc.	Mode of inoculation.	Initial weight in grammes.	Final weight.	Result.
Sputum I	No inoculation of cultures.		(Of sputum suspension) June 27, 96.		0.5	intra-abd.	672	430	Dies Aug. 11–12.
					0.5	sub-cut.	370	390	Chloroformed August 15 (active).
					0.5	intra-abd.	640	457	" July 20 (sick).
" II	3 mos. 3 days.	5th	Feb. 24, 97.	24	0.5	sub-cut.	570	520	Chloroformed April 19.
					0.5	intra-abd.	600		Dies March 6–7 (10–11 days).
	5 " 10 "	8th	May 1, 97.	9	0.5	" "	440	312	" May 13 (13 days).
" III	1 " 19 "	2d	April 3, 97.	18	0.5	" "	525		" April 12–13 (9 days).
	2 " 17 "	4th	May 1, 97.	9	0.5	" "	435	245	" May 17 (17 days).
" IV	2 " 16 "	2d	May 1, 97.	18	0.5	" "	705		" May 15–16 (15 days).
	6 " 10 "	7th	Aug. 26, 97.	9	0.5	sub-cut.	372	275	" Oct. 9.
	6 " 10 "	7th	Aug. 26, 97.	9	0.5	intra-abd.	507		" Sept. 16–17 (21 days).
" V	1 " 19 "	3d	Aug. 26, 97.	9	0.5	sub-cut.	458	402	Chloroformed Dec. 3.
					0.5	intra-abd.	517		Dies Sept. 5–6 (11 days).
" VI	1 " 12 "	3d	Aug. 26, 97.	9	0.5	sub-cut.	420	431	Chloroformed Nov. 11, extensive lung disease.
					0.5	intra-abd.	478	338	Dies Oct. 3 (38 days).
" VII	1 " 10 "	2d	March 1, 98.	16	0.5 *	sub-cut.	323	360	Dies May 14–15.
					0.5 *	intra-abd.	332	222	Dies March 18–19 (18 days).
Bovine I	15 " 19 "	14th	Mar. 20, 96.	16	0.5	sub-cut.	405	?	Chloroformed May 14 (failing).
					0.2	intra-abd.	478		Dies April 22 (33 days.)
" II	2 " 27 "	3d	Feb. 24, 97.	24	0.5	sub-cut.	580	?	" Mar. 24.
					0.5	intra-abd.	645	?	" Mar. 3–4 (7 days).
	5 " 3 "	5th	May 1, 97.	9	0.5	" "	505	?	" May 13 (13 days).
" III	4 " 7 "	5th	Aug. 26, 97.	9	0.5	sub-cut.	533	400	" Oct. 17–18.
					0.5	intra-abd.	624	?	" Sept. 2–3 (7 days).
	9 " 27 "	8th	Feb. 15, 98.	17	0.5	" "	581	468	" Mar. 2 (15 days).
" IV	4 " 3 "	5th	Aug. 26, 97.	9	0.5	sub-cut.	635	400	" Oct. 6–7.
					0.5	intra-abd.	533	?	" Sept. 4–5 (9 days).
" V	4 " 3 "	5th	Aug. 26, 97.	9	0.5	sub-cut.	504	336	" Oct. 5.
					0.5	intra-abd.	490		" Sept. 5–6 (10 days).
" VI	2 " 18 "	3d	Mar. 15, 98.	13	0.5 *	sub-cut.	406		Dies May 2–3.
					0.5 *	intra-abd.	423		Dies April 7 (23 days).
Nasua	19 " 22 "	18th	Mar. 20, 96.	16	0.75	sub-cut.	455	?	" April 26, probably chronic kidney disease.
					0.3	intra-abd.	505	?	" May 1 (41 days).
Swine I	2 " 6 "	14th	July 14, 96.	12	0.5	sub-cut.	525	?	" Aug. 7.
					1.0	intra-abd.	525		" July 25–26.
	11 " 23 "	16th	May 1, 97.	13	0.5	" "	450	300	" May 15 (14 days).
Horse I	5 " 4 "	6th	July 24, 97.	18	0.5	sub-cut.	395		" Aug. 19–20.
					0.75	intra-abd.	415	290	" Aug. 7 (14 days).
Cat I	4 " 15 "	5th	July 24, 97.	18	0.75	sub-cut.	424	290	" Aug. 28–29.
					0.5	intra-abd.	427	?	" Aug. 8 (15 days).

* Dilution one-third to one-fourth of that hitherto used.

Rabbits. The inoculation into the anterior chamber of the eye has been the most widely practised method. This seems to have been followed by variable results, whether due to the number of bacilli injected, the source of the bacilli or the amount of injury inflicted on the eye.

Koch injected tubercle bacilli from various sources into an ear-vein. The bacilli were suspended in blood-serum and the suspension passed through fine gauze to eliminate coarser particles. 4 rabbits were inoculated in this way with bacilli from a monkey, 3 with bacilli from a case of phthisis and 3 with bacilli from cattle. The rabbits all succumbed in from 18 to 31 days, excepting one, which was killed on the 38th day. In another experiment a culture from a case of lupus and from a monkey were tested on rabbits. Death ensued in 2 to 3 weeks. The weight of the rabbits and the density of the suspensions used are not given, so that comparisons are impossible. Moreover, the medium used—liquid serum—may not be entirely indifferent. Straus* mentions the slight effect produced upon rabbits by cultures of human tubercle bacilli.

In the present work, the injection of tubercle bacilli into an ear-vein was used at first tentatively, then, when sharp distinctions between bovine and human cultures were made manifest by this procedure, it was continued.

Rabbits weighing from 1300 to 2200 grms. were employed. The suspension was prepared as already described, and for most of the primary tests 0.5 cc. was injected. By referring to Table III it will be seen that for the primary test of the last pair of cultures, Bovine VI and Sputum VII, the suspensions were $\frac{1}{3}$ to $\frac{1}{4}$ as dense and the dose was doubled. The other cultures of which larger doses were injected are Sputum I, Nasua and Cat I (0.75 cc.), and Swine I (1 cc.). More recent tests indicate, however, that any variation in the dose as slight as these has but little influence in modifying the duration of the disease.

As far as practicable, the tests were made as soon as the purity of the cultures was beyond doubt, in order to reduce to a minimum the factor of artificial attenuation. This seems to have been on the whole incon-

* La tuberculose et son bacille, 377.

siderable.* An effort was also made to test the cultures in groups as far as possible, in order to furnish identical conditions. These groups are Bovine I and Nasua, Sputum II and Bovine II, Sputum III and IV, Sputum I and IV, Horse I and Cat I, Sputum V, VI and Bovine III, IV and V. Sputum VII and Bovine VI may be considered a group, though the inoculations were not made simultaneously, owing to an accident. The ages of the different groups of cultures varied from a minimum of 9 to a maximum of 24 days. The total age of the cultures likewise varied at the date of the first test from 1 month and 10 days to 9 months and 25 days. These differences, which were largely counterbalanced by the group inoculations, cannot be dwelt upon here, as the table contains all the information needed for the reader to form his own opinion. It should be stated, however, that the heaviest rabbits of any group were chosen for the bovine cultures. After the inoculation the rabbits were handled very little, in order that the conditions might remain as uniform as possible. For this reason I have no temperature records, but hope to obtain them from a special series of experiments.

The results may be classed as gross or those manifest on ordinary observation and those deducible from a microscopic examination of the diseased tissues and organs. The gross results show a sharp line of demarcation between the bovine and the human cultures. While all the rabbits inoculated with the former succumbed in from 17 to 21 days, of the rabbits inoculated with the latter only one succumbed in 35 days. The others were chloroformed after periods ranging from 1 month and 24 days to 3 months and 18 days. At the time they were killed all but one had increased in weight, some but little, others much.

The rabbits inoculated with bovine bacilli manifested in most cases more or less dyspnœa at the end of 8 or 10 days. The lesions were practically the same in all, with slight minor modifications. The lungs, as the first and chief recipients of the injected bacilli, were in a state of expansion, which in some cases was extreme. All lobes were densely sprinkled over with small grayish tubercles, varying from mere specks to those 1 mm. in diameter. The liver was usually mottled, indicating fatty changes, and in about half the cases it was permeated with barely visible grayish points. The spleen was enlarged to 2 or 3 times its normal size

* See Table III, Bovine culture I.

and as a rule permeated with minute tubercles. In the kidneys the visible tubercles were scarce. In 3 cases they were seen in the heart muscle, in several in the follicles composing Peyer's patch at the ileocæcal valve.

The sputum rabbits, with one exception, behaved quite differently from the bovine cases. A certain number showed signs of illness after 7 or more days. They were quiet, somewhat drowsy and ate little, but manifested no dyspnœa. This condition lasted one or two weeks, after which recovery took place. A certain number did not show this transitory depression. It is probable that with all, during the first month, there was loss of weight, for subsequent weighings showed no gain upon the initial weight. At the time they were chloroformed, they had all more than recovered in weight. The lesions in these rabbits were in many respects quite different from those of the bovine rabbits. The rabbit which received the culture marked Sputum I did not show any recognizable lesions. The difficulty experienced in the attempts to cultivate this bacillus leaves the interpretation of this case somewhat doubtful, although the microscopic preparation from the feeble culture used showed well-stained bacilli. The lesions of the other cases were quite uniform in character.

In all, the lungs contained tuberculous foci sprinkled over the various surfaces from 1 mm. to 1 cm. apart. They did not seem to interfere functionally, for the lungs collapsed normally when the thorax was opened. The tubercles frequently projected somewhat above the surface in the collapsed state of the organ. In the fresh condition the foci were observed as minute dots, barely visible owing to their translucency, as larger foci, 2 mm., in rare cases 3 to 4 mm. in diameter, also translucent excepting the centre, which was occupied by a minute opaque point, and as more diffuse infiltrations of a translucent appearance mainly at the sharp lateral margin of the lobes. These different forms of lesions were usually found associated in the same organ, the largest in the animals kept alive longest. Necrosis was an inconspicuous feature, whereas the translucency was often so perfect as to conceal the small lesions from the eye. When placed in alcohol, these became opaque and visible.

The liver usually contained minute tubercles scattered sparsely over the organ. The spleen, only very slightly larger than normal, was affected to nearly the same degree. The kidneys came next to the lungs in the extent of the lesions. Sprinkled sparingly over the cortex were opaque whitish foci from one to two mm. in diameter. When the organ was incised, these round foci, in many cases, corresponded to radial whitish lines and bands, extending from the periphery towards the pelvis and usually stopping within the medullary portion near its base. More

rarely small whitish foci were observed in the walls of the stomach or the large intestine. The lymph nodes were free excepting in one case, in which the node associated with a tuberculous kidney was involved.

In all cases inspection of the affected organs revealed no degenerative changes. They were evidently not disturbed by the focal lesions, an observation strengthened by the increased weight of the animals when sacrificed. The single rabbit which died 35 days after inoculation had received Sputum culture IV. It is worth while to note as a highly probable explanation of this one exception that the rabbit, after inoculation, escaped from the basket in which it was being carried and had to be chased for a long time in the animal room before it could be penned. I at once anticipated some modification of the disease, owing to the different distribution the bacilli would undergo under the influence of muscular exertion. This anticipation was realized. The rabbit after a week became quiet and remained dull and drowsy, without any change in respiration. On the day before death it sat in a crouching position, the hind feet drawn forward under the body, the eyes partly closed. After death its weight was 922 grammes, the initial weight having been 1620 grammes. During the latter weeks a purulent ophthalmia developed in the left eye, and shortly before death there was a slight discharge and beginning opacity of the cornea in the right eye.

In the liver, lungs and kidneys there were minute, translucent tubercles, scarce in the lungs and kidneys, more abundant in the liver. The lesions, found in sections of hardened tissue to be relatively very immature, could not explain the rapid emaciation and death, as they were less extensive than those of the surviving rabbits, unless we assume that the different, more general distribution caused a more rapid breaking up of the bacilli and diffusion of the toxines. Another hypothesis would be the presence of foci in the central nervous system, which was not examined. A second trial upon a rabbit 2 months later, with conditions as nearly the same as possible, proved no exception to the general rule. The rabbit showed but little disturbance, and was chloroformed after 2 months and 9 days. The lesions found were like those induced by the other sputum bacilli.

In order to probe still farther this difference between bovine and human bacilli, the lungs of all but a few rabbits were subjected to a microscopic study. Here the foci may all be considered primary and produced under like conditions. The lesions as defined in fixed and hardened tissue by the microscope in the bovine cases were characterized by rapid necrosis of the tubercle and the presence of very large numbers of tubercle bacilli. This necrosis was evident in the fresh organs from

the marked opacity of the comparatively young tubercles. The tubercle itself at the stage in which it came under observation varied slightly from case to case. It consisted of a necrotic centre, absent only in the smallest foci, outside of which a zone of epithelioid cells was distinguishable. This zone was in several cases bounded by a peripheral one of cells having deeply stained nuclei and probably lymphoid. These latter cells were also intermingled with the epithelioid ones. The central necrosis appeared in two forms; it either was characterized by the presence of a very dense, deeply stained mass of nuclear fragments, or else these were present only to a slight degree and the centre had a more rarefied aspect. In the latter case it was evident that the necrotic mass consisted of the bodies of epithelioid cells and the few nuclei present. In the former the source of the great mass of nuclear detritus remains in doubt. These dense nuclear masses were present in abundance in Bov. I and III. In Bov. II and IV they were not quite so conspicuous; in Bov. V least so. It is worthy of note here that Bov. I and III are the two cultures which proved rapidly fatal to cattle inoculated with them, as will be shown farther on.

In the series of rabbits inoculated with human bacilli, the lungs present quite different conditions. The lesions evidently develop very slowly, for in the one rabbit which succumbed after 35 days, the tubercles were very small, translucent, the bulk microscopic in size. With the continued life of the rabbit they grow larger. The microscopic picture differs from that of the bovine series in the rarity of necrosis and in the comparative scarcity of tubercle bacilli. The tubercles consist of aggregations of large epithelioid elements which only in the centre of the larger foci show necrosis. These epithelioid foci may be rarely enclosed by a zone of round cells. Giant cells are present, but as a rule in very small numbers. In the Sputum III rabbit they were exceptionally numerous and very large. The contiguous alveoli may be occluded with patches of epithelioid cells not to be confounded with desquamated epithelium.* Tubercle bacilli are found only in the necrotic centres, either in very small numbers or more abundantly. In some cases they stain feebly. In all cases they are somewhat longer than the bovine bacilli in the same situation.

In addition to the foregoing inoculation tests, made as far as practicable with equal doses, a few others were tried under slightly modified

*** The lesions in rabbits due to sputum bacilli resemble in many respects those produced by Prudden with boiled and washed tubercle bacilli in the same species, if we exclude the slight progressive tendency of the disease due to the living forms. See *N. Y. Med. Journal*, 1891, i, 637.**

conditions. A large rabbit (No. 20), weighing 2048 grms., received into the abdominal cavity the usual dose, 0.5 cc. of the standard suspension. Death ensued 26 days later. The walls of the abdomen and of the large intestine were densely studded with minute tubercles, the liver permeated with them. The lungs also were densely beset and penetrated with minute translucent tubercles. They did not differ appreciably from the lungs after intravenous inoculation. This mode of inoculation thus proved almost as promptly fatal as the intravenous injection. The same suspension of tubercle bacilli used upon the preceding rabbit was diluted with 9 volumes of bouillon, and 0.5 cc. injected into an ear-vein of a rabbit (No. 27) weighing 1932 grms. The rabbit thus received one-tenth the usual dose. In this animal the symptoms and duration of the disease and the lesions were nearly the same as those of the rabbits inoculated with the horse culture to be described later. The rabbit showed evidences of disease after two weeks, with slowly increasing dyspnœa. On the 34th day it was chloroformed, as it would probably have lived but one or two days longer. The extreme relative virulence of the bovine bacilli is thus put into a clear light.

A similar experiment was made with Sputum culture III. One rabbit received an equivalent of the usual dose, another one-tenth of this on March 1st. Both are still alive (July 20), and the initial weights of 1707 and 1672 grms. are now 1558 and 1986 grms.

It has already been stated that the Nasua culture, presumably derived from sputum, produced neither symptoms nor visible lesions in a rabbit. The swine and the cat culture, however, produced lesions practically identical with those due to the bovine cultures.

The horse culture takes a position midway between bovine and human cultures, so far as the rabbit inoculations show. These, therefore, deserve a more detailed description. The first rabbit, treated like all the rest, remained apparently unaffected for a month, although the weight had fallen slightly from 1505 to 1477 grms. In the second month respiration became somewhat slower and grew gradually more and more labored, so that the animal had to be chloroformed just two months after inoculation. The weight had fallen to 1232 grms. The lesions were macroscopically unlike those in any other rabbit of the series. In the first place, the lungs failed to collapse even slightly. They are densely beset and permeated with tubercles of a grayish, feebly translucent appearance, the majority about 1.5 to 2 mm. in diameter, coalescing into patches 4 and more mm. in diameter. Minute tubercles are also visible among the larger ones. The latter may have a minute, opaque, necrotic centre. The liver is apparently free from tubercles, but the slightly

enlarged spleen is beset with sharply outlined, slightly projecting grayish tubercles 1 mm. in diameter. The kidneys are affected in a manner similar to those of the sputum rabbits, but the foci are larger. There are scattering tubercles in the walls of the small and large intestines, but none in the walls of stomach. A second rabbit, inoculated 6 months later, was chloroformed after 40 days on account of severe dyspnœa. The course of the disease and the lesions were like those of the first rabbit. A noteworthy peculiarity of these cases is the presence of tuberculous foci 2 to 4 mm. in diameter in the superficial axillary and popliteal lymph nodes. These were not seen in any of the sputum rabbits, but were produced by retarding the bovine disease in a rabbit. The intermediate position of this horse culture is well illustrated by this experiment referred to above, in which a dilution of bovine tubercle bacilli, equivalent to one-tenth of the usual density employed, produced manifestly the same effect upon rabbits as the horse culture. In other words, the pathogenic power of the bovine culture as measured upon rabbits was 10 times that of the horse bacilli. Further trials will, perhaps, enable us to give a rough arithmetical ratio between the pathogenic power of the bovine and the sputum cultures as affecting adult rabbits. I do not, however, consider the difference between these races to be solely quantitative.

A comparison of the lesions produced by these three races was attempted by a histological examination of the lungs, spleen, liver and kidneys of three rabbits:

No. 16. Wt. 1527–1617: Sput. V: Chloroformed 3 months 7 days after inoculation.
No. 14. Wt. 1540–1232: Horse I: " 2 " 14 " " "
No. 19. Wt. 1443– Bov. V: Died 20 days after inoculation.

I refrain from giving the detailed account of the lesions in each organ and limit myself to the following summary:

Sputum V. Homogeneous epithelioid cell foci of small size, with very little central necrosis and very few tubercle bacilli. In the lungs and kidneys these foci have lymphoid cell mantles, in the liver well-defined connective tissue capsules.

Horse I. Large foci in lungs, where they are so numerous as to coalesce; some large foci in kidneys; smaller ones in the spleen. In the liver the foci are represented simply by one or several large giant cells, situated within the lobules. Foci large in subcutaneous lymph nodes and Peyer's patch. Necrosis of the foci quite extensive in all the organs excepting the lungs, where it is slight. Tubercle bacilli fairly abundant in all foci.

Bovine V. Large numbers of epithelioid cell foci in lungs, liver and spleen. In the latter epithelioid cells are also abundant throughout the pulp. Foci small in kidneys. Necrosis under way in lung foci. Tubercle bacilli very abundant in all foci.

Taking into consideration the time during which these lesions developed, we may epitomize the observed differences as follows:

In the first rabbit, very gradual development of the tubercles, with suppression of the bacilli and reparative changes leading to a stationary condition of the disease. In the second rabbit, continuous but not very rapid development of tubercles in the various organs. In the kidney and spleen evidences of limitation of the foci. No evidence that bacilli are repressed. In the third rabbit very rapid appearance of the foci, with unchecked multiplication of the bacilli and rapid onset of necrotic changes.

As a matter of some interest there may be briefly mentioned certain lesions of the eye in three of the sputum rabbits. In rabbit No. 7, inoculated with Sputum culture IV, which animal was the only one of all the sputum rabbits to succumb (in 35 days), a purulent ophthalmia appeared in the left eye some time before death. Soon after a slight cloudiness of the cornea of the other eye was noticed. A discharge had started in this shortly before death. I did not associate these lesions directly with tuberculosis, although later cases make it probable that the purulent discharge was secondary to tuberculosis of other parts of the eye. At the autopsy I examined the discharge for tubercle bacilli, but found no acid-resisting bacilli.

The second rabbit (No. 12) inoculated with a culture from the same source first called my attention to this localization of the tubercle bacilli. In this animal there was noticed some weeks after the inoculation, a slight nodular thickening of both lids of the right eye, which slowly grew more conspicuous. Vision became impaired by the enlargement and gumming together of the lids, so that the rabbit used the other eye only. When this animal was chloroformed, 3 flattish, disk-like, firm excrescences were found on the sclera at the margin of the cornea, about 90° apart. Of these, as well as of the nodulated lower eyelid, sections were made, and both lesions proved to be tuberculous. In both the tubercle bacilli were sparingly present. The neoplasm in the eye-ball had involved nearly the whole thickness of the sclera. In the eyelid the multiple foci were external to the lobules of the Meibomian glands. In still another animal eye lesions were found. About 2½ months after rabbit No. 16 had been inoculated with Sputum culture V, the lids of the left eye were observed to be gummed together. A pasty, yellowish

exudate was found under the lids and the cornea was slightly opaque. About 3 weeks later the animal was chloroformed and faint whitish spots were seen in both eyes, presumably on the iris. Sections of the eye hardened unopened in Zenker's fluid confirmed this supposition. Small epithelioid tubercles were found in the vascular stroma of the iris as well as in one ciliary body. Linear infiltration of the cornea was observed, starting from the most affected side and extending to the middle of the cornea. In its course several sections of vessels were found. A similar infiltration had begun from the opposite side. In the foci tubercle bacilli were very scarce. The infection probably came through the blood, as infection from the point of injection on the ear is hardly supposable, since the lesions here are of the slightest character. The animals had been kept separated, and could not have acquired the infection from the exterior. The eye of the rabbit thus seems to be a very vulnerable organ, and earlier experimenters have, in a way, chosen by accident that organ in this species most likely to give positive results.*

The divergences noticed when rabbits weighing between 1300 and 2200 grammes are inoculated into an ear-vein with 0.5 cc. of a well-clouded suspension of tubercle bacilli in bouillon are as follows:

1. Death of the bovine cases in 17 to 21 days.

2. Rapid evolution and necrosis of the pulmonary tubercles, with very great increase of the tubercle bacilli in them.

3. Death of the sputum rabbits (one explainable exception) did not ensue, but after $1\frac{1}{2}$ to $3\frac{1}{2}$ months the original weight had been more or less exceeded.

4. The pulmonary tubercles in the sputum rabbit developed very slowly, with very little tendency towards necrosis. The bacilli were present in very small numbers only.

5. The swine and cat cultures are to be classed with the bovine, the horse culture stands intermediate.

* The eyes of Sputum rabbit VII were affected in the same manner.

TABLE III.—Rabbits.

Culture.	Total age of artificial culture.	Number of transfer.	Date of inoculation.	Age of culture used, in days	Dose in cc.	Mode of inoculation.	Designation of rabbit.	Initial weight in grammes.	Final weight.	Chloroformed after	Died in
Sputum I	10 mos. 27 days.	2nd	June 16, 97.	61	0.75	ear-vein.	17	1470	1681	2 mos. 3 days.	
" II	3 " 3 "	5th	Feb. 24, 97.	24	0.5	" "	6	1380	1645	1 " 26 "	
	1 " 19 "	2nd	April 3, 97.	18	0.5	" "	8	1790	1830	2 " 8 "	
" III	13 " 17 "		Mch. 1, 98.	16	1.0‡	" "	28	1707		Alive July 20; weighs 1558.	
	13 " 17 "		" " "	16	1.0*	" "	26	1672		Alive July 20; weighs 1986.	
" IV	1 " 18 "	2nd	April 3, 97.	18	0.5	" "	7	1620	922		1 mo. 5 days.
	4 " ———	5th	June 16, 97.	12	0.5	" "	12	1620	1722	2 mos. 9 days.	
" V	1 " 19 "	3rd	Aug. 26, 97.	10	0.5	" "	16	1517	1617	3 " 7 "	
" VI	1 " 12 "	3rd	Aug. 26, 97.	10	0.5	" "	18	1305	2061	3 " 18 "	
" VII	1 " 10 "	2nd	Mch. 1, 98.	16	1.0†	" "	29	1882	1788	3 " 8 "	
Bovine I	15 " 19 "	14th	Mch. 20, 96.	16	0.5	" "	3	2279	——		17 days.
	37 " 24 "	38th	Jan. 25, 98.	12	0.5	" "	24	1795	——		22 "
" II	2 " 27 "	3rd	Feb. 24, 97.	24	0.5	" "	5	1360	——		17 "
" III	4 " 7 "	5th	Aug. 26, 97.	10	0.5	" "	15	1775	——		21 "
" IV	4 " 3 "	5th	Aug. 26, 97.	10	0.5	" "	17	1607	——		15 "
	9 " 23 "	9th	Feb. 15, 97.	17	0.5	abdomen.	20	2048	——		26 "
	9 " 23 "	"	" " "	17	0.5§	ear-vein.	27	1932	1416		Killed on 34th day, very severe dyspnœa, probably within 24 hours of time of death.
" V	4 " 3 "	5th	Aug. 26, 97.	10	0.5	" "	19	1443	——		20 days.
" VI	2 " 18 "	3rd	Mch. 15, 98.	13	1.0†	" "	21	2353	1877		Chloroformed on 22d day; dying.
Nasua	19 " 25 "	18th	Mch. 20, 96.	16	0.75	" "	2	1800	fat condition wt. ?	1 mos. 24 days.	
Swine I	2 " 6 "	4th	July 14, 96.	12	1.	" "	1	2000 (?)	——		17 days.
Horse I	5 " 4 "	6th	July 24, 97.	18	0.5	" "	14	1540	1232	2 " 4 "	
	11 " 5 "	11th	Jan. 25, 98.	12	0.5	" "	25	1653	——	1 " 9 "	
Cat I	10 " 17 "	5th	Jan. 25, 98.	18	0.75	" "	13	1320	——		20 "

* Dilution about one-twentieth of the standard. † Dilution about one-fourth of the standard. ‡ Dilution about one-half of the standard. § Dilution about one-tenth of the standard.

Gray Mice. White mice have been pronounced quite insusceptible by Koch. Of 5 inoculated subcutaneously with a culture from the monkey, 12 with a culture from miliary tuberculosis, only one of the first set showed a few grayish nodules in the lungs. On the other hand, field-mice were found quite susceptible.

Two parallel tests were made upon gray house-mice with cultures of Bovine V and Sputum VI. At the time control inoculations of the suspensions used were not made upon guinea-pigs, and subsequent developments made it doubtful whether Bovine V was alive. This part of the experiment is therefore omitted from the table given below and a later experiment with Bovine VI substituted. The suspension used in this latter test was employed simultaneously upon 2 guinea-pigs and a rabbit with the usual success.

In the first bovine test the 4 mice inoculated, 2 subcutaneously and 2 into the abdomen, all died, the first on the 8th and the last on the 37th day, without any lesions recognizable with the naked eye. In the cases of the 4 mice inoculated with the sputum culture, death ensued between the 8th and the 40th day. In one of these the lungs contained a number of whitish, rather firm, nodules, 1 to 2 mm. in diameter. In sections these were found to consist of completely necrotic foci, enveloped by a dense, broad zone of pneumonic infiltration, within which all alveoli were filled with nuclear debris. A striking feature were the immense numbers of rather slender, mainly beaded tubercle bacilli, which occupied not only the necrotic centre, but in equal abundance the pneumonic periphery. This case proved the vitality of the sputum culture, and the test is therefore inserted in the table below.

TABLE IV.—Gray Mice.

Designation of culture.	Total age of culture.	Number of transfer.	Age of culture used.	Dose and mode of injection.	Results.
Sputum VI	5 mos. 27 days.	6th	24 days	Mouse 1, 0.5 cc. subcut...	Dies in 34 days, no tuberculosis.
				" 2, 0.5 cc. " ...	Dies in 40 days, no tuberculosis.
				" 3, 0.25 cc. abdomen.	Dies in 8 days, no tuberculosis.
				" 4, 0.25 cc. "	Dies in 34 days, tubercles in lungs.
Bovine VI	2 mos. 18 days.	3rd	13 days	" 5, 0.25 cc. subcut..	Chloroformed in 37 days
				" 6, 0.25 cc. " ..	" " "
				" 7, 0.25 cc. abdomen	Dies in 5 days.
				" 8, 0.25 cc. "	" " 5 "

The second bovine test resulted in the death of the two mice receiving the dose into the abdomen, in 5 days. There were no lesions recognizable. The two mice inoculated subcutaneously were active up to the 37th day, when they were chloroformed. Lesions were not found.

Pigeons. Fowls and pigeons have been used with success in distinguishing avian from mammalian tubercle bacilli. Hence it was thought best to test human and bovine bacilli on pigeons. The cultures used are given below in the table. The bacilli, in the form of bouillon suspensions, were injected into the pleuro-peritoneal cavity, the needle being introduced caudad of the sternum. In one case the injection was made into a wing-vein. The inoculations were in general negative, with the possible exception of that of the swine culture. In this bird there was found hyperæmia of the serosa of the intestines, with an eruption of scattering, minute grayish dots. There were similar opaque points on the air-sac membranes. On the duodenal loop were found some particles of viscid exudate composed exclusively of coarsely granular cells. In this exudate some tubercle bacilli were still recognized by the specific stain. In some of the other pigeons there were a few opacities of the mesenteries and air-sac membranes, with perhaps one or more conspicuous Peyer's patches. Even in refractory animals, lesions such as these might be anticipated where the tubercle bacilli are deposited.

TABLE V.—Pigeons.

Designation of culture.	Total age of culture.	Number of transfer.	Age of culture used.	Designation of pigeon.	Dose and mode of injection.	Remarks.
Sput. II	6 mos. 14 d.	9th	11 days	No. 2	1 cc. abdomen...	Chloroformed in 45 days: negative.
" III	3 " 19 "	5th	11 "	" 4	1 cc. " ..	Chloroformed in 57 days: negative.
Bov. II	5 " 26 "	9th	11 "	" 1	1 cc. " ..	Chloroformed in 49 days: negative.
Swine I	1 yr. 19 "	17th	11 "	" 3	1 cc. " ..	Chloroformed in 57 days: minute excrescences on portions of intestine and membranes of air-sacs.
Sput. V.	5 mos.	5th	31 "	" 5	¾ cc. wing-vein..	Chloroformed in 47 days: negative.
Bov. V	8 mos. 14 days	8th	24 "	" 8	1.5 cc. abdomen..	Chloroformed in 49 days: negative.
Horse I	11 mos. 5 d.	11th	12 "	" 7	0.5 cc. "	Chloroformed in 50 days: negative.

Cattle. It is a noteworthy fact that, in spite of the great economic, agricultural and hygienic interests involved in the diffusion of bovine tuberculosis throughout the world, no experiments upon cattle with cultures of the bovine bacillus had been made up to the time of my first experiments, published in the *Transactions of the Association of American Physicians* for 1896. Experiments upon cattle with the human bacillus had been made by several observers. A discussion of these tests I shall defer to the end of this article, because of certain secondary questions, which will be clearer when my tests have been described.

The results of the inoculation of two heifers into the lungs with Bovine culture I and *Nasua* culture have already been published.* It may be stated briefly that the bovine culture produced general tuberculosis, ending fatally in 35 days, while the Nasua (human) culture failed to produce any recognizable lesions.

During the warm season of 1897 inoculations were made with 5 sputum, 1 swine and 4 bovine cultures upon as many cattle. For these 10 animals I am indebted to the Massachusetts Board of Cattle Commissioners, who have shown much interest in the progress of these investigations, because of the need felt by them of more definite information upon the relation of bovine tuberculosis to the human disease. The test was carried out in two series, the first including 4, the second 6 animals. The culture material used was suspended in bouillon, as described, the density of the suspension being about the same as that of a typhoid culture in bouillon 24 hours old. In both series the dose was 2 cc. of such a suspension.

Series I. The 4 animals of this series were inoculated with the following cultures:

Sputum II,	total age	5	months	9	days;	8th	transfer.
Sputum III	"	2	"	15	"	4th	"
Bovine II,	"	5	"	2	"	5th	"
Swine I,	"	11	"	23	"	16th	"

The swine culture is probably bovine in origin, and it was used on this supposition. Assuming that tubercle bacilli become slowly weakened

* Loc. cit.

in virulence by artificial cultivation, we should endeavor to use cultures as fresh and of as nearly the same age as possible. This theoretical demand cannot be successfully met, because of the many difficulties surrounding such work. Of the 4 cultures used, Sputum II and Bovine II are of nearly the same age, while Sputum III is but half as old as they, and Swine I more than twice as old.

In making the injection, the space between the 6th and the 7th rib was chosen. The needle was inserted about 3 inches above the level of the elbow (olecranon process). It was found subsequently, at the autopsy, that the point chosen was too low, and that in all of the animals the needle, leaving the lungs intact, pierced the diaphragm. Some of the bacilli were discharged into the muscles of the thoracic wall, some into the abdominal cavity and some into the pleural cavity. Bearing this accident in mind, we may now go to a description of the further history of the inoculated cattle. They were all housed in a spacious, well-ventilated barn in large commodious horse stalls. A piece of ground adjoining the barn was enclosed, and in this the animals spent 6 to 7 hours a day for about 4 weeks. Thereafter the animals were separated into two lots, one lot being out several hours in the morning, the other several hours in the afternoon. The two which received the bovine and the swine culture were allowed to run together, similarly the two which received the sputum cultures. It might be claimed that there was in this arrangement a possible danger of transmitting the bacilli from one animal to another and of infecting the ground. There was no evidence of this at the post-mortem examination, and the arrangement was considered safe at the start because it takes some time for the tuberculous tissue to become disintegrated. It was, however, deemed prudent not to keep the animals longer than 2 months, on account of the imperfect isolation.

In apportioning the cultures to the animals, the sputum cultures were injected into the youngest animals, in order that these cultures might have any advantage likely to accrue from difference in age. Two yearlings (without any permanent incisor teeth) received the two sputum cultures, the bovine culture was injected into a heifer about 2½ years old, the swine culture into a heifer about 2 years old.

They were killed and examined at the Brighton (Boston) abattoirs, with the co-operation of the Board of Cattle Commissioners, just two months after the day of the injection. Before injection, these animals had been tested with tuberculin by the Board and found free from tuberculosis. Still, since this agent occasionally allows an animal to escape which contains foci of the disease, attention was directed to this point

at the autopsies. No lesion, however, was found which from its situation and appearance could be referred to any former spontaneous infection.

Let us examine first the effect of Sputum culture II and Bovine culture II, which were of nearly the same age when injected.

The weight of the yearling which had received the sputum culture had risen in the two months from 520 to 580 lbs., that of the heifer from 650 to 710 lbs. There was no continuous fever recognized in either animal, though the temperature was taken twice a day, morning and afternoon. The fluctuations noticed were evidently due to the effect of the sun while the animals were in the enclosure.

The lesions in the yearling were very slight. At the seat of inoculation between the 6th and 7th ribs a mass of tubercles attached to pleura about 1 x ½ inch in dimension, the tubercles composing it partly cheesy, partly firm. Near the cephalic border of ventral lobe of right lung, a subpleural nodule, not yet necrotic, about ⅛ inch in diameter. On abdominal aspect of diaphragm, right side, about 24 isolated tubercles $\frac{1}{12}$-⅛ inch in diameter. A few similar tubercles on the omentum, which is slightly adherent to the large intestine. When adhesion was removed, about 6 or 7 nodules found on caecum $\frac{1}{12}$ inch in diameter. Evidently the injection needle had passed through the diaphragm into abdomen, depositing some fluid there and some in thorax when partly withdrawn.

The lesions in the heifer which had received the bovine culture were quite extensive and were diffused through thorax and abdomen, owing to the penetration of the diaphragm by the needle of the injection syringe:

Thorax. **The right pleural cavity shows an abundant eruption of tubercles along the borders of the ribs. Some of the masses formed are characteristically flattish, grape-like and in bulk quite large. One mass measured 8½ x 3 x 1½ inches. Others of similar dimensions were present. On the lateral margin of the right lung a series of loosely attached, flattish neoplasms up to 2 inches in diameter, besides hyperæmic fringes of loose connective tissue. On the convex surface of this lung only a few tubercles. Large patches of tubercles on pericardium and diaphragm.**

In the muscular portion of diaphragm, right side, a mass of tuberculous tissue (probably place where needle penetrated) 1½ x 1½ x ½ inch in dimension. The caudal of the series of dorsal mediastinal glands about twice normal size on account of the presence of many small foci, showing in some cases an opaque, yellowish centre.

Abdomen. The omentum densely studded with agglomerations of tubercles covering the greater part of its surface. These masses vary up to ½ inch in thickness. Similar patches on abdominal aspect of diaphragm and on spleen. Fewer patches on gall-bladder and on liver. In one of the portal glands a ¼-inch focus, pale, grayish, permeated with small calcareous spicules.

The examination of sections of the pleural eruptions and of one of the dorsal mediastinal glands showed the typical lesion of bovine tuberculosis, with its foci of epithelioid cells and its numerous giant cells containing a few tubercle bacilli. Any detailed description of these lesions would be simply a repetition of familiar facts.

We have in these cases a wide divergence in the result of inoculation. The human bacillus produced a slight eruption of small, tubercle-like bodies, which did not present even microscopically the characters of true tubercles, while the bovine bacillus produced an exquisite case of pearly disease both in thorax and abdomen, with the formation of large, grape-like masses in the chest.

The youngest sputum culture (III) was injected into a yearling weighing 410 lbs. At the end of two months the weight had risen to 480 lbs. The lesions found are slight:

On the abdominal aspect of diaphragm, right side, a patch of isolated tubercles about 2 inches in diameter, the tubercles themselves about $\frac{1}{8}$ inch in diameter and about $\frac{1}{2}$ inch apart. They are grayish, opaque. At place of inoculation, on serous aspect of ribs, a flattish neoplasm 1 inch in diameter and about $\frac{1}{3}$ inch thick. Other lesions not detected. In this case also the needle evidently entered abdomen through diaphragm. Sections made from the small isolated tubercles on the abdominal surface of the diaphragm show them to be masses of newly forming tissue. They consist in the main of spindle-shaped connective-tissue cells running in parallel and interlacing groups and richly supplied with vessels. No trace of the characteristic tubercle formation or of bacilli, although these bodies are evidently due to the bacilli injected.

The swine culture was injected into a somewhat older animal weighing 620 lbs. After two months the weight was 660 lbs. The autopsy showed the following conditions:

At place of inoculation (right chest wall) a subcutaneous tumor about 2 inches in diameter, made up of a very dense connective-tissue sac, ¼ inch thick, which encloses a pale yellowish semifluid mass (caseous). There are besides this focus, in the same situation, 3 smaller nodules from ½ to ¾ inch in diameter, the largest with caseous centre. On the pleural aspect a similar ½-inch centrally softened focus.

In nearly the centre of the right half of diaphragm and projecting into abdomen for ½ inch is a tumor representing portion of a larger focus in the muscular portion of the diaphragm about 1 inch in diameter. This focus is likewise caseous, diffluent centrally. On all the ribs of right pleural cavity are eruptions of small tubercles, reaching in some instances a diameter of ⅛ inch. Besides the palpable tubercles, there is along one border of each rib a line of vascular fringes of connective tissue. In many of these fringes tubercles are not noticed, although the fringes themselves are evidently a result of the injection.

Along the lateral border of the right lung similar vascular fringes of tissue containing tubercles. These fringes extend dorsad for about 2 inches on ventral and cephalic lobes.

Diffuse eruption of minute tubercles on pleural aspect of diaphragm, right half.

Large caudal mediastinal gland contains large numbers of tubercles, varying in size from mere points to those ⅛ inch in diameter and showing beginning necrosis. Left bronchial gland several times normal size. Ventral mediastinal gland several times normal size and containing several large, centrally caseous foci. One mesenteric gland contains several small necrotic tubercles.

In this case many of the bacilli had been deposited in the fleshy portion of the diaphragm and some under the skin, and had thus been prevented from exerting their greatest power. Sections were made of the subcutaneous foci, the anterior mediastinal and bronchial lymph-glands and of the vascular fringes on the pleural aspect of the diaphragm. The glandular and subcutaneous lesions are characteristically tuberculous, with a greater tendency towards necrosis and disintegration of the tubercles than in the animal inoculated with Bovine culture II. The lesions of the serous membranes are more vascular and show greater tendency toward the formation of connective tissue around the minute tubercles. In general this culture must be considered less potent than the bovine culture. Whether this is due to the more prolonged artificial cultivation or to the species through which the bacillus had passed remains unsettled.

Series II. In this experiment three sputum and three bovine cultures, recently isolated, were used. The bovine cultures were of nearly the same total age. The sputum cultures differed somewhat from them and from one another in this respect.

Sputum	IV,	total age	6	months	10	days,	7th	transfer.
"	V,	"	1	month	19	"	3rd	"
"	VI,	"	1	"	12	"	3rd	"
Bovine	III,	"	4	months	7	"	5th	"
"	IV,	"	4	"	3	"	5th	"
"	V,	"	4	"	3	"	5th	"

The cultures used for the injection were all 9 days old, grown on the same lot of dog's serum. Control inoculations of guinea-pigs and rabbits will be found on tables II and III. The animals at my disposal, unfortunately, varied considerably in age, and in assigning the cultures the advantage was given to the human cultures:

Sputum IV, yearling, 525 lbs., no permanent incisor teeth.
Bovine III, " bull, 645 lbs., no permanent incisor teeth.
Sputum V, cow, 675 lbs., about 4 years old.
Bovine IV, " 850 " " 12 "
Sputum VI, cow, 865 lbs., about 3½ years old.
Bovine V, " 875 " " 6 "

The injection of the cultures was carried out as in Series I, excepting that the point of insertion of the needle was chosen higher up, about 11 inches above the elbow of the animal when in the standing position, between the 7th and 8th ribs. The length of the needles used was about 2 inches. The care of the animals was the same as that bestowed on the preceding lot, excepting that the bovine and the sputum animals were kept separate in the out-door enclosure from the start, the one lot being out in the morning, the other in the afternoon. They were kept two months, with the exception of the young bull, which died 17 days after the inoculation. The four cows of this lot gave at the start altogether about 8 quarts of milk. The secretion was slowly dried up, so that in the sixth week a very little, amounting perhaps to one quart in two days, was removed. The milking was continued chiefly to prevent any udder troubles during the experiment and to maintain normal conditions. The temperature was taken but once a day, at noon.

In comparing the temperature records of these 6 animals it was noticed that the three animals which received the bovine cultures had a high temperature immediately after the inoculation, which lasted until the death of the bull and about three weeks for the remaining two animals. At the same time no such elevation of temperature was recorded for the animals receiving the three sputum cultures. There was but one well-defined rise of temperature in the case of the yearling, from the 13th to the 16th day after inoculation. The other irregularities are probably due to the fact that the temperature was usually taken after these animals had been in the enclosure in the sunshine for several hours. Those with the high temperature were kept much of the time in the cool barn in the morning, which probably depressed the fever curve somewhat. After the period of fever, no other elevations were noted up to the close of the experiment.

The young bull, inoculated with Bovine culture III, showed besides the prompt onset of a high temperature, general and local disturbances about a week after the inoculation. The breathing became rapid, the appetite had partly gone. Emaciation and weakness supervened. He was unable to get up September 11, and died the following night.

The autopsy revealed a severe miliary tuberculosis of both lungs with marked congestion and œdema of the organ. Normal collapse no longer possible. The associated lymph-glands were much enlarged and infiltrated with minute tubercles. Patches of minute tubercles were found on the pleural covering of ribs and on the omentum. Sections from the lungs, dorsal mediastinal glands, pleural neoplasms, liver, spleen and kidneys were examined. In the lungs, mediastinal glands and pleural neoplasms, tubercles were very numerous, in the liver less abundant. In the kidney sections none were seen. In these different situations the tubercles presented nearly the same peculiar features, which distinguished them readily from the tubercles of the spontaneous disease in cattle. To make the differences clearer, a brief description of the lesions in a spontaneously diseased lymph-gland may not be out of place.

A rarefied area within the follicle containing a few nuclei having the characters of endothelioid nuclei and belonging to what are generally known as epithelioid cells, which are imbedded in or whose outlines are fused with a matrix appearing either homogeneous or else very delicately reticulated. In larger foci this rarefied area contains also one or more giant cells whose body is frequently seen merging by prolongations into the reticulated matrix of the tubercle. Among the epithelioid nuclei are isolated nuclei closely resembling those of lymphoid cells. Tubercle bacilli are, as a rule, scarce and situated usually within giant cells. The largest foci are centrally necrotic, that is, they contain a coarse trabecular matrix staining with eosin and picric acid and containing scarcely any nuclei and very little, if any, fragments of nuclear origin. In this mass tubercle bacilli are rarely found. In the lesions of the bull the tubercles have in the first place no giant cells. The new tissue appears to be made up of cells resembling the usual epithelioid cells of tubercle, but much more crowded together and with less definition of the cell outlines. The nuclei are mostly distorted, drawn out, band-like or horse-shoe-like, and twisted into various bizarre forms. Condensation and fragmentation of the nucleus are also seen. In the tubercles in all situations much fibrin is demonstrated by Weigert's stain. This is especially distinct in the liver tubercles. Here the intercellular substance from material hardened in alcohol stains deeply in eosin. In sections subjected to Wei-

gert's fibrin stain, this substance is blue, appearing throughout the tubercle both as a delicate mesh and as very coarse trabeculæ. In material hardened in Zenker's fluid the fibrin stain is less pronounced. In all the tubercles, large numbers of tubercle bacilli are present. These differences are probably due to the rapidity of the process.

On October 27, the remaining five animals were killed at the Brighton abattoirs, with the co-operation and assistance of the Board. The three sputum animals had all gained in weight:

Yearling (Sputum IV) from 525 to 610 lbs.
Cow (Sputum V) from 675 to 750 lbs.
Cow (Sputum VI) from 865 to 960 lbs.

Sputum culture IV. One permanent incisor on the right has appeared since date of inoculation. In utero a fœtus about three months old. At point of inoculation in the subcutaneous tissue a small nodule about ¼ inch in diameter with contents soft, cheesy. Attached to this is another, smaller nodule about ⅛ inch in diameter.

On the right side of chest wall, pleural aspect, there are attached along the six caudal ribs, soft dark red, pendulous masses of newly-formed, highly vascular connective tissue. At point of inoculation, between 7th and 8th ribs, a flattish pediculated mass of tissue of brownish red color. On the 10th rib another mass about ⅜ inch in diameter. The left side of thorax is normal.

Right lung. On the smaller (cephalic and ventral) lobes, newly formed, delicate fringes of hyperæmic connective tissue, which appears also along the free lateral margin as a delicate band about ½ inch broad, as well as on a portion of the convex surface of the lung, occupying exclusively the lines representing the boundaries of the lobules.

In the large caudal lobe, which is similarly beset with the vascular fringes, a tumor, representing the place where needle penetrated the lung tissue, projecting slightly above the convex surface, is found two inches from the caudal tip. This tumor, about ¾ inch in diameter, contained a completely disintegrated mass and about a dozen surrounding foci, $\frac{1}{16}$ to ⅛ inch in diameter, with yellow softened centres. On the margin of this same lobe, in addition to the vascular fringes, are four firm masses of grayish tissue, smooth, flattish, attached by pedicles to the margin of the lobe. The diameters of the largest are ¼ and ½ inch.

On large (caudal) lobe of left lung there is only a very little development of vascular fringes. Imbedded in the same lobe near lateral margin is a uniformly grayish, slightly translucent mass, sharply defined from the enveloping normal lung tissue. Sections of this focus showed a peculiar lymphoid structure of the tumor, in the centre of which a parasite was lodged. Not

having any relation to the inoculation disease, it need not be further discussed.

Attached to the cephalic lobe of the left lung by a pedicle is a small, flattish, smooth mass of new tissue.

The pleural aspect of diaphragm and portions of the pericardium are covered with areas of the highly vascular neoplastic tissue. In some, small nodules can be felt at the free extremity of this tissue.

On the right ventricular surface of the heart four flattish, pediculated masses about as large as split peas are attached.

The various lymph-gland systems, ventral and dorsal mediastinal and bronchial glands, do not show the presence of tubercles or any augmentation in size.

Of this case, sections of the primary lung focus, of two of the pediculated bodies (one from the lung, one from the rib) and of the vascular fringes from the lateral border of the lungs were examined.

The small foci immediately bordering the disintegrated primary focus vary from 0.2 to 2 mm. in diameter. The smallest do not differ essentially from ordinary tubercles, excepting in the scarcity of giant cells. The larger ones are centrally necrotic, with a marked tendency towards encapsulation on the periphery by the condensation of circularly disposed, fusiform, connective-tissue cells. In the smallest tubercles, bacilli are scarce, in the largest they are numerous within the necrotic centre, where they appear as slender, often bent, sometimes fragmented and rather feebly stained rods. In the surrounding epithelioid zone a few well-stained bacilli are present. Passing from the primary place of deposit, the only other lesions are those of the pleura. These occur in two forms, as vascular fringes and as firm, smooth, pediculated bodies, some flattish, like pumpkin seeds in size and outline.

The vascular fringes occur: 1. As mere endothelial tubes; 2. As such enveloped by a layer of wavy fibrillar tissue and covered with a layer of endothelial cells; and 3. As vessels sheathed with a zone of round cells, proliferating endothelial and connective-tissue cells in addition to the fibrillar tissue. Acidophile, polymorphous leucocytes are present here and there throughout the granulation tissue. Mitoses are occasionally seen. After a prolonged search through 7 sections, one minute rarefied area is detected. In it evidently a tubercle bacillus with long axis nearly at right angles to the section.

The vascular fringes from the thoracic aspect of the diaphragm are like those from the lungs. In the former no signs of tubercle formation.

Sections from one of the neoplasms attached to the ribs show two fairly distinct regions, an outer of dense connective tissue and an inner made up of foci of lymphoid elements and a central necrotic core. This latter body was evidently a vascular offshoot of the pleura, which, before necrosis, had attached to or imbedded in it many lymphoid cells, for the necrotic core contains much nuclear matter traceable to such cells. This core is partly envel-

oped by a ring of epitheliod cells with nucleus at the outer free end, like those around a foreign body. Some are multinuclear and resemble giant cells. In the zone immediately outside of this cellular ring are several characteristic giant cells. There are no tubercle bacilli, nor is there any tissue recognizable as epithelioid and identifiable as tubercle.

Sputum culture V. Cow about 4 years old. In utero a fœtus 3 to 4 months old.

Development of vascular fringes along one border of ribs of the right side of thorax as in preceding case, but amount relatively slight. In the intercostal muscles at the point of inoculation a mass of perhaps a dozen small grayish tubercles.

In the large caudal lobe of the right lung, in the same situation as in preceding case, a projecting tumor about 1 inch in diameter. When incised it is found composed of two ½-inch foci of disintegrated, cheesy-viscid matter enclosed in thin, smooth capsules. No surrounding infiltration. Along the margin and on the caudal surface of this lobe, slight development of pendulous vascular tissue and a sessile tubercle about 4 mm. in diameter. On the left caudal lobe only very slight production of vascular tissue.

On the surface of one of the middle dorsal mediastinal glands an aggregation of minute tubercles, the whole about ½ inch in diameter. Imbedded in the cortex of the same gland two minute tubercles.

In this animal the disease of the middle mediastinal gland, although very slight, is shown in sections to be tuberculosis. The lesions are not to be distinguished from those of the spontaneous disease in cattle, excepting in the presence of considerable numbers of tubercle bacilli. The tubercles themselves contain the usual number of giant cells, and the largest ones are centrally necrotic, the necrosed mass being indistinctly fibrillar in character, staining deeply with eosin and devoid of nuclear debris. The bacilli in this mass stain rather feebly when compared with the deep stain of those in the giant cells on the periphery of the tubercle. This lesion will be again referred to in summing up the experiments.

Cow (Sputum culture VI). In utero a fœtus about four months old.

Within the thorax on the right side, between the 7th and 8th ribs, is a small, pediculated, blackish (hæmorrhagic) mass of firm tissue about as large as a pumpkin seed, another on the 10th rib. On most of the ribs behind the 7th are gelatinous-looking pendulous vascular fringes of neoplastic tissue. On the pleural surface of the diaphragm a similar development of vascular tissue and several firm, pediculated masses like those on ribs.

Of the right lung the cephalic lobe shows very slight formation of marginal fringes. In the ventral lobe whose tip is adherent to the pericardial fat, a uniformly grayish, sharply defined focus ¼ inch in diameter.

In the same situation as in the preceding cases the caudal lobe shows a slightly projecting tumor about 1¾ inches in diameter externally. When incised it is found to consist of a smooth-walled sac 1¼ inches in diameter, containing a yellowish curdy mass, together with a little turbid fluid. No surrounding infiltration. On the convex surface of this lobe there is a slight growth of vascular tissue. Near the caudal tip a flattish mass, partly yellowish, partly blackish, attached by loose tissue to the margin of the lobe. It is about 1 inch long and ½ inch thick.

In the abdomen, a flattish, sessile mass of pinkish-gray tissue about ¾ inch in its longest diameter, attached to omentum.

In this animal, sections of the grayish focus in the ventral lobe of the right lung and of the suspicious sessile mass on the omentum demonstrate the fact that both lesions are parasitic in origin and due to the same cause as the lung focus in cow inoculated with Sputum IV. Both foci are made up of spherical agglomerations of lymphoid cells, in the centre of which the parasite is situated.

The largest of the pendulous masses from the lungs was examined microscopically and found to be similar in structure to those already described. It consists chiefly of a connective-tissue stroma of interlacing fibres in which are imbedded many capillaries. Scattered through the inner half of the mass are quite small foci of epithelioid and lymphoid cells, but giant cells are absent. In these foci, a few tubercle bacilli are found. The periphery of this pendulous mass consists of circularly condensed connective tissue, beneath which is a homogeneous, somewhat reticulated substance, staining well with eosin and enclosing a few tubercle bacilli. It is probably a layer of fibrin undergoing hyaline degeneration. Small areas of it are still stainable with Weigert's fibrin stain.

Of the three animals receiving bovine tubercle cultures, the fate of one (young bull No. 71) has already been given. The two other cases remained stationary in weight:

No. 88—original weight 850 lbs.; weight at end of experiment 850 lbs.

No. 63—original weight 875 lbs.; weight at end of experiment 870 lbs.

The autopsy notes are in brief as follows:

No. 63 (Bovine culture V). White cow, spotted with red. Horns sawed off. Probably six years old. Fœtus in utero about two months old.

Thorax. Right lung adherent to chest wall in several places. At point of inoculation, between 7th and 8th ribs, an excrescence of the costal pleura about ¾ inch in diameter, of dense, pearly-looking connective tissue enclosing a disintegrated mass. Numerous masses and aggregations of small tubercles on all ribs. These masses in some cases are several inches in length. The left side of thoracic wall, below the level of the point of inoculation on

the opposite side, is covered with a uniform, pinkish-gray deposit of very minute tubercles. Eruptions of tubercles on pleural surface of diaphragm and on pleural covering of dorsal mediastinal space.

On caudal lobe of right lung a considerable number of tubercular masses, flattish, sessile, from ⅛ to ¾ inch in diameter. Between the cephalo-lateral border of this lobe and the pericardium is a mass of newly formed tissue, dense, in which are imbedded many minute yellow tubercles and masses of pericardial fat. The whole is about as large as a fist. It binds the lung tissue, pericardium and diaphragm together. Many tubercles on the caudal surface of this same lobe. There is no distinct focus in this lobe as a result of the injection, and it is probable that much of the fluid was deposited in the pleural cavity. But palpation reveals throughout both lungs small shot-like bodies in close proximity. On section numerous yellow tubercles from $\frac{1}{32}$ to $\frac{1}{16}$ inch in diameter are found imbedded in the lung tissue of all lobes.

The dorsal mediastinal lymph-glands are all several times their normal dimensions. They contain many coalescing yellow tubercles. The ventral (anterior) mediastinal glands are similarly enlarged and the cut surface shows a uniformly cheesy parenchyma.

Minute grayish points under the capsule of the liver.

In the spleen, all Malpighian bodies converted into tubercles with yellow opaque centre. In left kidney several minute grayish tubercles.

Of this case, sections of the left lung, left costal pleura, the large caudal mediastinal gland and the spleen were examined.

The tubercles from the lungs do not differ from those found in spontaneous tuberculosis. The centre is as a rule necrotic and contains a few tubercle bacilli. Outside of this is a zone of nuclear fragments and entire nuclei, shading off into a peripheral zone of epithelioid and lymphoid cells and cells with spindle-shaped nuclei. Giant cells are present in nearly all the tubercles. The new growth on the left side of the thorax consists essentially of newly formed vascular fringes, in which are imbedded minute tubercles with scarce bacilli and occasional giant cells.

In the caudal mediastinal gland the tubercles vary from a mere giant cell to those 2 mm. in diameter and centrally necrosed. The latter are like those in the lung tissue. The bacilli in some necrotic foci are numerous, in others few in number.

In the spleen the follicles contain epithelioid cell foci, some of which are centrally necrosed and which contain many bacilli.

No. 88 (Bovine culture IV). Red and white cow. Teeth very much worn, probably 12 years old, dehorned. Not pregnant.

No deposit in subcutis at point of inoculation. The eruptions of tubercles on the costal pleura of the right and the left side are in character very much like those of the preceding case (No. 63), but less extensive.

Right lung. Lobes adherent to pericardium. Adhesions readily severed. On convex surface of the caudal lobe of this side a considerable number of flattish sessile tubercles from $\frac{1}{16}$ to $\frac{1}{2}$ inch broad. The caudal aspect of this lobe is similarly beset with them, but in less abundance. Along the margin of this lobe are loosely attached, small, elongated masses of tubercles.

At the same situation in this lobe as in the sputum cases, there is a fluctuating tumor, about two inches in diameter, slightly projecting. It consists of a capsule with nearly smooth walls enclosing a soft caseous mass. It is surrounded by a zone of small necrotic tubercles and with lobules containing numerous minute, grayish foci.

On the cephalic lobe of the right lung are a considerable number of grayish tubercles. Throughout all lobes are scattering tubercles in the lung tissue, some very minute, others larger and opaque, yellowish in color.

The pleura in the dorsal mediastinal space is beset with a large number of small tubercles; similarly the pericardium. The right half of the diaphragm is beset with flattened aggregations of tubercles. Between the ventral lobe of the right lung and the pericardium, and fastening them together, is a mass of newly formed connective tissue and fat enclosing numerous softened foci.

The large dorsal mediastinal lymph gland is enlarged and contains a large number of yellow tubercles. The central portion of the gland is uniformly caseous. In the left bronchial gland a small number of tubercles; in the ventral mediastinal glands a considerable number. Organs of the abdomen appear free from tuberculous changes. Of this case only the tubercles disseminated through the lungs were examined microscopically. For this purpose the left lung was chosen. The tubercles are small, compact up to 1 mm. in diameter. In structure they are indistinguishable from the spontaneous bovine tubercles. The bacilli in them are few in number, rather short, thick and deeply stained.

A summary of the outcome of this last experiment may now be made. The points of difference between the inoculation disease produced by bovine and by human (sputum) bacilli are several.

1. The bovine cases either remained stationary in weight or lost slightly, while the sputum cases gained 75 to 85 pounds. Still, the age of one of the bovine cases may be partly responsible for stationary weight.

2. There was marked fever in the bovine cases for three weeks after the inoculation; practically none in the sputum cases.

3. There were well-marked differences in the lesions produced. In the sputum cases the lesions are nearly the same and consist in:

(*a*) A tumor in the right caudal lobe of the lung, about 1 inch in diameter, projecting somewhat above the surface of the lung. This represents the place where the needle penetrated into the lung tissue and deposited the tubercle bacilli. In each case the contents of this tumor were softened and converted into a curdy mass, enclosed in a thin-walled capsule, smooth internally. The disease was not spreading from this point nor were tubercles visible in the lymph glands of the lungs and thorax, excepting in one gland of No. 39 (Sputum V).

(*b*) The free margin of the right lung and the pleural covering of the ribs on the right side were beset with a new formation of loose vascular fringes or shreds in which in only one case some minute nodules could be felt, and some flattish, pediculated masses, which did not resemble tubercles at all histologically, or which were only abortive forms of the same.

Among the bovine cases we have the following characteristic points to note:

(*a*) Disseminated tuberculosis of the lungs, severest and fatal in No. 71, the youngest; least pronounced in the oldest, No. 88. Associated with this a local disintegrated focus in the lungs of No. 88.

(*b*) Tubercular deposits on lungs, pericardium, diaphragm and the ribs, resembling closely the product of the natural disease in cattle. Extension of the eruption to the costal pleura of the left side.

(*c*) Extensive tuberculosis of all or nearly all the lymph glands of the thorax, including both mediastinal chains.

(*d*) Slight tuberculosis of other organs, spleen, liver, kidney, in two out of three cases.

A summary of the three separate tests on cattle (including the published experiment), in which twelve animals were used, shows that 6 animals were inoculated with human bacilli, 5 with bovine bacilli; 1 animal was inoculated with swine bacilli.

Of the sputum cases, 1 showed no disease; 2 showed very slight lesions; 3 showed only local lesions without dissemination.

Of the bovine cases, 2 died of generalized disease; 2 showed extensive lesions; 1 showed less extensive lesions.

In the swine case the lesions were less extensive than in the bovine cases.

TABLE VI.—CATTLE.

Designation of culture.	Total age of culture.		Number of transfer.	Age of culture used.	Amt. of suspension of bacilli injected.	Designation of animal.	Age, etc.	Original weight in pounds.	Final weight in pounds.	Date of inoculation.	Result.
	mos.	days		days							
Bovine I	5	4	3rd	10	4 cc.	No. 284	heifer, 2½ yrs. old, common stock, pregnant.	(?)	(?)	May 4, 1895	Died in thirty-five days of generalized tuberculosis.
Nasua narica	9	9	8th	10	4 cc.	No. 300	heifer, 2½ yrs. old.	(?)	(?)	May 4, 1895	Killed June 27, 1895, no lesions.
Sputum II	5	9	8th	9	2 cc.	No. 2616	yearling.	520	580	May 1, 1897	Killed July 1, 1897, lesions very slight.
Sputum III	2	15	4th	9	2 cc.	No. 2634	yearling.	410	480	May 1, 1897	Killed July 1, 1897, lesions very slight.
Bovine II	5	2	5th	9	2 cc.	No. 2635	heifer, 2½ yrs. old.	650	710	May 1, 1897	Killed July 1, 1897, extensive pearly disease in thorax and abdomen.
Swine I	11	23	16th	13	2 cc.	No. 2672	heifer, 2 years old.	620	660	May 1, 1897	Killed July 1, 1897, well-marked pleural tuberculosis with invasion of lymph glands.
Sputum IV	6	10	7th	9	2 cc.	No. 79	yearling, about 1½ years old.	525	610	Aug. 26, 1897	Killed Oct. 27, 1897, abscess in lungs at point of injection, new vascular tissue on ribs and lungs.
Sputum V	1	19	3rd	9	2 cc.	No. 39	cow, 4 years old.	675	750	Aug. 26, 1897	Killed Oct. 27, 1897, lesions same as preceding.
Sputum VI	1	12	3rd	9	2 cc.	No. 76	cow, 3 years old.	865	950	Aug. 26, 1897	Killed Oct. 27, 1897, lesions same as preceding.
Bovine III	4	7	5th	9	2 cc.	No. 71	bull, yearling.	645	575*	Aug. 26, 1897	Died Sept. 12, 1897, disseminated tuberculosis of lungs, liver.
Bovine IV	4	3	5th	9	2 cc.	No. 88	cow, about 12 years old.	850	850	Aug. 26, 1897	Killed Oct. 27, 1897, many minute tubercles in lungs, tuberculous deposits on pleura.
Bovine V	4	3	5th	9	2 cc.	No. 63	cow, about 6 years old.	875	870	Aug. 26, 1897	Killed Oct. 27, 1897, disseminated tuberculosis of lungs and spleen.

* Two days before death.

ON THE SIGNIFICANCE OF VARIETIES AMONG TUBERCLE BACILLI.

The foregoing experiments, while they show unmistakably the close relationship existing among the various cultures studied, nevertheless justify us, if only to guide and stimulate further study, in establishing a distinctively human or sputum and a bovine variety of the tubercle bacillus. It might be better to omit the host designation of such varieties, in order to anticipate assumptions that they are necessarily limited to the host whose name they bear. Still, the convenience of using the host's name is so great that I shall succumb to it. The characters upon which the bovine variety may be based reside, morphologically, in the invariably short, straight form and in the greater resistance of this form to modifying influences of culture-media; biologically, in a greater resistance to artificial cultivation and in a much greater pathogenic activity towards rabbits, guinea-pigs and cattle.

There is proof, furthermore, of the existence of slightly varying characters even within the varieties proposed. Among the bovine forms studied, slight variations in virulence were noticeable. Among the sputum forms, variations in size, in capacity for cultivation, and in pathogenic activity have been observed. The differences between Sputum culture I, on the one hand, and Sputum cultures II to VII, on the other, are quite pronounced. In spite of these variations, mammalian tubercle bacilli may still be considered as forming a fairly compact group when compared with the tubercle bacilli of birds, which are but slightly virulent towards the guinea-pig, so susceptible to the mammalian type.*

In regard to the bovine and the sputum varieties, I have, in a former paper, pointed out certain differences of behavior in the body which may be of considerable significance in explaining their divergent characters. In an organism like the human body, manifesting a certain amount of resistance to the tubercle bacillus, the sputum type multiplies only to a limited extent, unless it can produce dead tissue or gain

* I have, unfortunately, been unable to study avian bacilli in connection with the series of this article, because opportunities for making cultures from fowls were wanting. The study of cultures of unknown or indefinite history I have not regarded as likely to yield trustworthy evidence.

an exit upon mucous membranes secreting pathological products, where it seems to flourish as a saprophyte. Similar conditions are now and then observed in animals inoculated with human bacilli. Immense numbers of bacilli are occasionally found in necrotic foci, but very few in the surrounding zone of living tissue. In the many sections examined recently, the necrotic foci were the ones in which human bacilli were to be found, if detected at all. The bovine type differs from the former in a far less saprophytic growth. In the pathological secretions and in the caseous masses the bacilli are relatively scarce. This difference may be a result of their adaptation to the bovine body, in which cavities of the lungs and catarrh of the air tubes are far less common. In other words, certain differences in the type of reaction tend in the one case to make the human bacillus more saprophytic, the bovine more parasitic.

Coming to the more practical aspect of this subject, we may present, in view of the relationship between the bovine and the sputum variety of the tubercle bacillus, two propositions for discussion:

1. The sputum bacillus is incapable of finding a foothold in the bovine body.

2. The bovine bacillus may pass to the human subject, owing to its higher pathogenic power.

The transmission of human tuberculosis to cattle has been experimentally essayed by Bollinger, Crookshank, Baumgarten, Sidney Martin, the writer and Frothingham.

Bollinger * reported in 1894 an experiment performed in 1879. A calf three months old was inoculated into the peritoneal cavity with fluid from a tuberculous human lung. The calf was killed in 7 months. On the mesentery and the peritoneal covering of the spleen there were found pediculated tumors from the size of a pea to that of a walnut. The microscopic appearance corresponded completely with that of the spontaneous pearly disease of cattle. The retroperitoneal and mesenteric glands were tuberculous. All others were normal. The little drawing which Bollinger published with the article gives one the impression of smooth, roundish tumors similar to those I have described as resulting from the injection of Sputum cultures IV and VI. They have not the

* *Münchener med. Wochenschr.*, 1894, No. 5.

nodulated appearance presented by the spontaneous eruptions or those produced by bovine cultures.

Crookshank * injected sputum containing numerous tubercle bacilli into the peritoneal cavity of a calf. The calf died after a prolonged illness on the 42nd day.

> Extensive lesions were discovered at the post-mortem examination. The mesentery was adherent to the rumen; the liver was adherent to the diaphragm. There was extensive tubercular deposit at the seat of inoculation and an abscess the size of a walnut. Extending over the mesentery from this point there were hundreds of wartlike, fleshy new growths, some quite irregular in form, others spherical or button-shaped. There were similar deposits on the under surface of the liver, on the spleen, in the gastro-splenic omentum and on the peritoneal surface of the diaphragm. The spleen was adherent to the rumen, and on dissecting away the adhesions, another abscess was opened. On the under surface of the liver was a third abscess about the size of one's fist, which burrowed in the depth of the liver substance.
>
> On microscopic examination, extremely minute tubercles were found disseminated throughout the lungs and liver. Tubercle bacilli were found in these organs and in the peritoneal deposits. The pus from the liver abscess contained streptococci. The calf died of pyæmia, a result to be anticipated if sputum be employed for inoculation, but sufficient time had elapsed for pronounced local infection leading to acute miliary tuberculosis.

I quote this experiment more fully so that it may discredit itself as a means of deciding whether sputum can produce general miliary tuberculosis in cattle. We know as yet too little of the influence of concurrent infections to place any reliance upon the outcome of this experiment as a demonstration of the transmissibility of human bacilli to cattle.

Baumgarten † briefly mentions an experiment in which human bacilli in cultures had but little local effect after intraocular inoculation upon a calf. Material from a bovine pearly nodule produced typical local and general miliary tuberculosis in another calf.

Sidney Martin ‡ fed one kilogram of tuberculous material from a cow to 4 calves at one meal with their food. Within 4 weeks tubercles were found in the Peyer's patches, mesenteric and bronchial glands and in the lungs of one animal. After 8 weeks, another calf presented the same lesions plus tuberculosis of the posterior mediastinal and the cer-

* *Trans. Pathol. Soc.*, London, 1891, 332.

† *Jahresbericht* for 1891, footnote, 666.

‡ Report of the Royal Commission on Tuberculosis, 1895. Appendix, p. 18.

vical glands. The third calf, killed after 12 weeks, showed ulceration of the small intestine and cæcum and lesions of the pleura, besides those mentioned above. The fourth calf, killed after 37 weeks, had tuberculosis of the pleura in addition to that of the various lymph glands already mentioned.

Four calves were fed at one meal with 70 cc. of sputum containing a large number of tubercle bacilli. Three calves, killed after 4, 8 and 12 weeks respectively, had 53, 63 and 13 nodules respectively in the small intestines, chiefly in Peyer's patches. In the fourth calf, killed after 33 weeks, no lesions were found.

Two calves received at one feeding 440 cc. of sputum, containing a large number of tubercle bacilli. In one calf, killed in 8 weeks, 13 tuberculous nodules were found in the intestine. The mesenteric glands were also affected. The other calf, killed in 19 weeks, was found without any lesions.

The author infers from these experiments that "in the case of tuberculous sputum we are dealing with material which is less infective to calves than bovine tuberculous material."

Frothingham * carried out some experiments of a similar character for the Mass. Cattle Commission. A culture of human bacilli was used, isolated from the liver of a child and about 1 year old. Two calves, 3 months and 3 weeks old respectively, received suspensions of the culture into the peritoneal cavity. In both only slight local nodules were produced, some resembling spontaneous tubercle, others tending towards granulation tissue. Two calves, 3 weeks and 2 months old respectively, were inoculated into the trachea. In one case the large local abscess in the muscles of the neck indicated a deposit there of much of the material destined for the lungs. In the liver and lungs a small number of minute tubercles, devoid of tubercle bacilli, were found. In the other calf lesions were absent. Thus, in spite of the immature age of these animals, the tubercle bacillus may be said to have had but a trifling local effect on them. The tests on guinea-pigs indicate a very attenuated culture. Three additional calves inoculated subcutaneously with sputum containing many tubercle bacilli showed lesions equally slight.

The defect of most of these tests is the absence of parallel experiments with bovine tubercle bacilli. Inasmuch as we do not yet know anything concerning the relative resistance of cattle to tuber-

* Report of Mass. Cattle Commission for 1897. See also *Zeitschr. f. Thiermed.*, 1897, 330.

culosis at different periods of life, nor the relative effect of cultivation on the bacilli and of different modes of inoculation, such parallel experiments are quite essential. Of these few experiments, Bollinger's is the only one which might be regarded as favoring the susceptibility of very young cattle to human bacilli. Even here the disease penetrated only to the mesenteric and retroperitoneal lymph glands.

A summary of the inoculation experiments made by the writer upon cattle have already been given. Here we need simply to report the most salient features. These are—

1. The purely local, restricted character of the lesions in cattle due to sputum bacilli. Lesions were not found beyond the place of deposit or in the lymphatic glands to which these are tributary, excepting in one animal (Sputum V culture). In this case we find a multiple tubercular focus measuring *in toto* about 1 to 1.5 cm. in one of the dorsal mediastinal glands. This is the only affected gland found in the 5 cases. Considering the fact that the bacillus was one of the more attenuated, that on the other hand the animal was fully 4 years old, I am strongly inclined to look upon this isolated instance of lymph gland infection as a latent infection with bovine bacilli which was probably favored by the depressing effects of the injection into a slight activity. I take this ground because of its isolated occurrence and the very typical bovine character of the lesions. It is not unusual for such a slight lesion to be encountered in animals which have failed to react towards tuberculin, for experimenters agree upon a margin of error of 5 to 10 per cent, the lesions found in such cases being usually quite insignificant. Had the culture used not shown a virulence not only not above but slightly below the average virulence of the other sputum bacilli, as tested on a rabbit and on guinea-pigs, the above inference could not be justly drawn.

2. The atypical tissue changes induced by the sputum bacilli which tended chiefly towards granulation tissue. Tubercle formation recognizable as such was uncommon. Besides the lymphatic focus cited above as of doubtful origin, tubercles approaching nearest the bovine type were encountered in the primary lung focus in the youngest animal (Sputum IV).

Some writers, including Bollinger and Baumgarten, are inclined to regard the pediculated masses produced on serous membranes by the inoculation of sputum or of cultures (Sputum IV, VI) as something specific and as deciding positively the transmissibility of human bacilli to cattle. Leaving aside the abortive, atypical, microscopic structure of the pediculated masses produced in the writer's series, and assuming that the inoculation of young calves may furnish lesions of a more typical character, we may present anatomical and histological facts which go far to show that there is nothing characteristic or peculiar about them or about pearly disease in general.

On the omentum and the pleura of cattle not tuberculous there may exist in many cases patches of delicate filamentous fringes, probably in all cases the result of some local inflammation. These fringes are microscopic in size and only visible to the naked eye when they are present in large numbers and congested. My attention was first called to them in the study of Texas fever. Owing to the congestion of the whole portal system, these fringes, massed together, appeared as red patches on the omentum. The microscope showed them to consist of a capillary encased in a sheath of delicate fibrillar tissue and the latter covered by the endothelium of the peritoneum. Recently I have demonstrated the same fringes in slightly raised, reddened patches of the costal pleura in otherwise healthy cattle. Here they are quite inconspicuous, but may be demonstrated by seizing the surface of the membrane with delicate forceps. Invisible threads hold the latter near the membrane. In the fresh condition their structure is like that of the fringes on the omentum. In some I was unable to demonstrate a capillary. In all cases they consist of the wavy fibrillar tissue devoid of nuclei, but containing at times a few granule cells, recognized as acidophile leucocytes in stained sections. In sections, these fringes could be traced from their attachment to the serosa. The fibrillar tissue is directly continuous with the subserous layer of like tissue and the endothelial layer of the pleura is continuous over these fringes. The immediate union of fringes containing a capillary with the pleura was not demonstrated in the limited number of sections examined. Besides these of the simplest structure, others are seen in an active state of proliferation and infiltrated with lymphoid and acidophile leucocytes.

These fringes or filaments, probably the result of traumatic and other inflammation, form an excellent place of attachment for the injected tubercle bacilli. Here processes of epithelioid and giant-cell formation go

on rapidly when bovine bacilli are present, whereas the sputum bacilli tend to lead only to granulation tissue, with slight abortive tubercle formation. These new formations, subject to pressure and friction, may undergo hæmorrhage, anæmic necrosis due to twisting of the pedicle, and subsequent increase of the connective tissue stroma around the dead fringe. In this way those peculiar smooth nodes probably arise which are briefly described under the cases inoculated with Sputum cultures IV and VI. It is, furthermore, evident from the inoculation experiments that, among other causes, the tubercle bacillus itself is one inducing the formation of granulation tissue. The highly vascular, velvety layers of granulation tissue carpeting the costal pleura in most of the cattle inoculated is a sufficient proof of the existence of this tendency. The pearly disease may thus be a product of at least two factors, the presence of this tissue as a result of former proliferation, and the inherent tendency of the serous membranes of cattle to respond by the formation of these vascular fringes to tubercles situated under the pleura and to bacilli discharged subsequently from such tubercles. But still other factors may be concerned.

That a certain retarded movement of the disease process may produce pearly disease in other animals has been answered in the affirmative for rabbits by Troje and Tangl.* These authors inoculated subcutaneously bacilli from cultures and material from the tuberculous lungs of rabbits which had been intimately mixed with iodoform and kept in the dark in contact with it for one or two weeks. In two rabbits, from two separate experiments, chloroformed respectively 7 and 9 months after inoculation, pediculated tubercles of the serous membranes were found. Those not containing angular concrements, and therefore sectionable, were peculiar in the possession of a very large number of very large giant cells.

These observations I am able to confirm by one case, the rabbit inoculated with the culture of Sputum VI. This rabbit was chloroformed 3½ months after the intravenous injection of tubercle bacilli. Its weight had risen from 1305 to 2061 grms. The notes on this case are briefly as follows:

Organs apparently unaffected (brain and cord not examined), excepting the liver, kidney and lungs. The liver contains a small number of 1 to 2 mm. centrally caseous foci. The kidneys show a considerable number of grayish opaque 1 mm. tubercles. The lungs collapse well. They are everywhere beset with translucent bodies, seen with some difficulty, owing to their glassy

* *Deutsche med. Wochenschrift*, 1892, 191.

appearance. Some have a minute, point-like, opaque centre. In the small cephalic lobes there are larger confluent patches, especially on the margins, of a peculiar grayish-translucent appearance. Sections of hardened tissue show the distinctly epithelioid character of the tubercular changes, with very few giant cells. Necrosis only in the largest foci. Tubercle bacilli fairly abundant in the necrosed centres, elsewhere scarce. From the marginal tubercles finger-like prolongations, precisely like those of the bovine pleura, project into the pleural cavity. They consist mainly of rapidly proliferating connective-tissue cells imbedded in a very delicately fibrillar meshwork. The basal portion of the fringe is partly covered with endothelium continuous with that of the pleura and contains a number of capillaries in transverse section. Similar vascular fringes appear on the opposite margin of the same section.

It is not improbable that if this animal had been allowed to live as long as those of Troje and Tangl, tubercle bacilli would have found their way into these fringes and produced the so-called pearly disease. This rabbit was allowed to live longer than any other of the sputum series. We may thus assume that neoplasms of the pleura in rabbits may be looked for after the third month. These observations go far to show that the eruption of tubercles on the pleura is not so much an expression of peculiarity on the part of the tubercle bacillus as it is an expression of a certain grade of virulence of the bacillus in an organism possessing a certain grade of resistance to it. To these conditions may possibly be added a pronounced tendency in certain animals towards production of granulation tissue of a certain type on serous membranes.

Troje * has also described a rare case of pearly disease in man, affecting the pleura. Here, possibly, the previous existence of outgrowths like the fringes on the serous membranes of cattle may have been responsible for the peculiar localization of the tubercle bacillus.

Putting all the facts obtained by experiments upon cattle together, it would seem as though the sputum bacillus cannot gain lodgment in cattle through the ordinary channels. These avenues, well provided with protective mechanisms, receive the bacilli probably one at a time. However closely the sputum and the bovine bacillus may be related, it seems as if under ordinary circumstances the former would fall an easy prey to destruction. This inference will gain in weight if we bear in mind that the far more potent bovine bacillus produces in at

* Loc. cit.

least 50 per cent of the spontaneously infected cattle a purely local disease, which probably would remain so if the animal were surrounded with favorable conditions.

The second and most important proposition, the transmission of bovine bacilli to the human subject, has been much discussed in recent years, without, however, bringing us any nearer to definite knowledge.

A recent compilation of cases of presumable transmission from cattle to man, both through wounds of the skin and through the digestive tract in milk, by Ravenel,* gives perhaps the most condensed account up to date. To these may be added a most interesting case reported by Coppez.† A girl 17 years old had a wound on the palmar aspect of the third finger between the 2nd and 3rd phalanx, which became infected with tubercle bacilli during milking. The original lesions gave rise, within 6 months, to over 35 subcutaneous abscesses situated in different parts of the body. There were two on the right hand, four on the elbow, two on the shoulder-blade, several on the right cheek, two on the left palm, one on the back of the neck, a dozen on the buttocks and thighs, four on the left leg, three on the sole of one foot and one on the big toe. Most of these were curetted, and iodoform applied 6 months after the beginning of the disease. Subsequently more appeared, in all from 60 to 66. The author describes the appearance of these foci as follows:

At a certain point, always in the neighborhood of a pre-existing focus, a thickening of the subcutaneous tissue appeared, associated with increased heat of the skin and pain on pressure. The heat soon disappeared, and a fluctuating livid tumor opened itself and discharged pus and later a yellowish serum. These various abscesses healed slowly, and within a year all had disappeared. At no time could any visceral lesions be recognized, but one eye became involved. The nature of the affection was demonstrated by inserting some pus from an abscess into the eye of a rabbit. There was but slight reaction at first. Subsequently the whole eye became diseased, the lymph nodes of the neck being greatly enlarged. After several months death ensued.‡

* *Journal of Compar. Medicine and Vet. Archives*, December, 1897.

† Un cas de tuberculose cutanée et oculaire sans manifestations viscérales, *Rev. gén. d'ophthalm.*, xv (1896), 433.

‡ In an article written just before the discovery of the tubercle bacillus, Creighton (Bovine tuberculosis in man, *Journ. Anat. and Physiology*, xv (1880), 1-177) describes 12 cases, following one another quite closely in the same hospital, which he considers to be afflicted with the bovine disease. This type of tuberculosis was characterized by eruptions on the serous mem-

If bovine bacilli may invade the human body without let or hindrance, we have not only food infection through milk and milk products to guard against, but also the inhalation disease to which men are exposed in stables containing tuberculous cattle. What proportion of tuberculous subjects may derive their infection from these sources we do not know. Now that we have established some fairly pronounced differences between bovine and sputum bacilli, the whole discussion might be cut short by the suggestion that the time has come to stop citing old and doubtful cases and to go to work to study with care the tubercle bacilli from cases of supposed animal origin, so that some experimental, trustworthy basis may be formed upon which to found statistics. While this is in truth what will have to be done and is the goal which has been aimed at from the outset in this tedious work, it will take much time and persistent attention to collect evidence of this kind. In the meantime the relation of bovine to human tuberculosis must be somehow defined before a fairly helpless and frightened public. It seems to me that, accepting the clinical evidence on hand, bovine tuberculosis may be transmitted to children when the body is overpowered by large numbers of bacilli, as in udder tuberculosis, or when certain unknown favorable conditions exist.* To prevent this from occurring, a rigid, periodic dairy inspection and the removal of all suspicious udder affections and all emaciated animals is as much as public health authorities can at present demand. Any measures beyond these belong to agriculture, with which the public health has no business to meddle, without endangering the chances of

branes, large, grayish-white medullary tumors in the lungs, frequently assuming the form of a wedge, and by certain microscopic peculiarities. These cases may have been due to some other organism. Quite recently Askanazy (Ueber tumorartiges Auftreten der Tuberkulose, *Zeitschr. f. klin. Med.*, xxxii (1897), 360), besides giving a good bibliography, cites 2 cases in full in which the peculiar type of disease leads him to suggest their relation to pearly disease in cattle. These articles simply impress upon the reader that the true interpretation of such cases will remain obscure until the etiological agent has been studied. The variable character of the tissue reaction to the tubercle bacillus, according to the species, deprives the pathological study, taken alone, of any decisive value.

* Probably much the same as in glanders. The number of glandered horses in certain places is out of all proportion to the cases in men who are continually handling and cleaning them.

gaining authority to enforce its own necessary measures. If the evidence gained by pathology in the future should reveal a greater danger than is here assumed, the scientific basis of such evidence will, I think, force all additional measures needed.

But for the student of etiology the problem does not end in the differentiation of varieties. It reaches out much farther than this and involves some puzzling questions. The most important one bears on the possible changes which the tubercle bacillus may undergo during its prolonged sojourn in the human body. I have already referred to one phase of this question in mentioning the saprophytic growth of the sputum bacillus in the affected lungs and necrotic tissue, as contrasted with its slight multiplication in living tissue and with the generally slight multiplication of the bovine bacillus in the tissue of cattle. This question is a very complicated one, and nothing is easier than to reason in a circle about it, because of the entire absence of data. The first hypothesis to be considered is that which assumes the conversion of the bovine bacillus into the sputum bacillus in the human body. This hypothesis would deny us all possibility of utilizing different characters of tubercle bacilli in tracing their source. If the bovine variety may enter into the digestive tract as such, and after a more or less prolonged interval emerge from the secondarily affected lungs as the sputum variety, we may as well give up all further study of the tubercle bacillus. The accumulated evidence of bacteriology is opposed to this view. Nevertheless, it has been unwittingly assumed by Klebs, in considering intestinal infection with the bovine bacillus, as a common cause of tuberculosis.* As a support of the view that tubercle bacilli change but very slowly, I may again mention the inoculation test upon a rabbit of Bovine culture I. About three years after the first test, the second test resulted in only a very slight prolongation of the disease. The first rabbit succumbed in 17, the second in 22 days. It was thus after 3 years of artificial growth still far above sputum bacilli in pathogenic power.

The question of phthisis as secondary to infection by way of the digestive organs is, however, one needing more attention, for experi-

* Die kausale Behandlung der Tuberkulose, 1894, p. 34.

mental results in this direction are quite suggestive. In all mammals the lungs are evidently the most favored place of tubercle bacilli, and wherever the latter may be deposited, they sooner or later, unless the disease is checked, reach that organ, where the process spreads more rapidly than elsewhere. This march from the place of infection is not infrequently partially concealed by reparative processes. A recent case in point is that of a guinea-pig inoculated with a culture of Sputum VI:

Aug. 29, 1897. Male guinea-pig, weighs 420 grams. Receives subcutaneously in the flank 0.5 cc. of a clouded suspension of tubercle bacilli (Sputum VI) 9 days old.

Nov. 8. Breathing now labored; has been active since inoculation.

Nov. 11. Respiration labored and almost convulsive; hence chloroformed; weight 431 grams.

No ulcer at point of inoculation.

Kneefold glands of this side as large as peas, yellowish in color, consisting of a thin-walled sac and homogeneous contents, resembling thick cream. Kneefold glands of opposite side moderately enlarged, but without necrotic changes. The pelvic, renal, retrogastric and mesenteric glands enlarged slightly, but without necroses. Spleen small, shows grayish specks disseminated through it. Liver and kidneys appear normal. Two small tubercles on left testicle.

Lungs do not collapse. They feel firm everywhere. On section most of the lung tissue is consolidated, of a grayish-white color. No fluid expressible from air tubes. In sections of the lungs, the infiltration consists chiefly of epithelioid cells with smaller, more deeply stained nuclei intermingled or in patches. No necrosis. The liver was, through an oversight, not preserved. The spleen shows in each follicle a rarefied area occupied by epithelioid cells and a few tubercle bacilli. No necrosis.

In this susceptible animal the only lesions threatening life or even conspicuous were those in the lungs. Those in the spleen were slight, possibly stationary. The disintegrated superficial glands might have discharged outwardly later on and healed. This is but one of a number of cases I have observed in the past 10 years in which there was progression in the lungs with almost complete destruction of the

organ and retrogression elsewhere, showing itself in the liver by shrinking and furrowing of the organ.*

With the two facts before us that tubercle bacilli gravitate, as it were, towards the lungs in all the susceptible mammals, and that they may conceal their movements in the body quite effectually, we must regard infection through the digestive tract as a source of phthisis at least deserving more attention. The only question to interest us here is the relation of the bovine bacillus to this process. Only much painstaking work will enable us to learn whether the human body can produce such a great modification of the bovine bacillus or not.

Another more acceptable hypothesis concerning changes in the tubercle bacillus would assume a slow, continuous modification which in the past has developed the sputum variety on the one hand and the bovine variety on the other. The future may enable us to establish not only other varieties linked to certain animal species, but also varieties which produce slightly different forms of disease in man himself.† The saprophytic life of the sputum bacillus in phthisis may be the cause of a very slow downward descent of its invasive power, which in the course of one or more generations may become perceptible both to the bacteriologist and to the clinician, and express itself to the statistician in the gradually falling curve of tuberculosis, as shown by the vital statistics of the past 40 years.‡

The outcome of such slow changes would lead to the establishment of numerous minor varieties because of the great opportunity which tubercle bacilli have in our congested, abnormal city life of selecting their variously predisposed victims. Thus the more attenuated varieties may find their invasion obstructed in the more vigorous, but not

* This generalization has been recently emphasized by Spengler (*Ztschr. f. Hygiene*, xxvi (1897), 321) as follows: The retarded course of an inoculation tuberculosis expresses itself without exception in a (greater) prominence of the lung disease with simultaneous retardation of the liver and spleen affection.

† Recently I have been able to study cultures of tubercle bacilli from a rapidly progressive tuberculous gland of the neck in an adult. These bacilli are slightly less virulent than the sputum bacilli described.

‡ S. W. Abbott. The Vital Statistics of Massachusetts. Rep. of the State Board of Health for 1896, p. 787-8.

in children or certain predisposed individuals. It is needless for me to illustrate these possibilities still farther. They have their parallels in nature all about us. Only one practical suggestion may be made, in view of the probable discovery of minor varieties of human bacilli. Tuberculous patients should not be indiscriminately exposed to other tuberculous patients simply because both are affected with a disease which bears the same name.*

A more exhaustive experimental study of the tubercle bacillus away from the many variable factors acting upon the human body, which will lead to a better standardizing of virulence or invasive power, may help these speculations to find some substantial basis. That much is still to be learned concerning the etiology is evident from the want of unanimity as regards the channels of infection. The views of Koch and his followers of the transmission of the infection in dust and spray would tend to favor the hypothesis of gradual attenuation and of the production of minor varieties. Baumgarten's hypothesis of the transmission of the tubercle bacillus from parent to child before birth implies the transmission of tubercle bacilli either in a continual struggle with living tissue or else remaining in situations where the bacilli cannot multiply, and hence where a tendency to loss of invasive power by saprophytic growth is impossible. The occasional entrance of bovine bacilli into the human body might open the way for the introduction of a virus of a higher level, provided opportunity for subsequent transmission be afforded.

* A beginning in this comparative study of different clinical types of tuberculosis has been made by Auclair (*Arch. de méd. exp.* (1897), ix, 1124). This article, which came to my notice after the completion of this manuscript, describes 4 cultures, one from a rapidly progressive tuberculosis of a gland of the neck, one from a slow phthisis, one from an acute meningitis and one from true scrofula. On guinea-pigs the intra-abdominal inoculation of these cultures had nearly the same effect. Hence the author did not hesitate to regard them as identical in pathogenic power, in opposition to the views of Arloing, who considers the bacilli of phthisis and those of the surgical forms of tuberculosis of different virulence. Auclair did not inoculate rabbits, in spite of the fact that Arloing regarded them as the best means of differentiating the bacilli of the different forms of tuberculosis. My own results show that if the intra-abdominal inoculation of guinea-pigs is the only test made, practically no differences worth considering would appear between bovine and sputum bacilli, as regards pathogenic power.

If in this brief summary I have presented nothing but problems to be solved and doubts to be entertained, I feel quite confident that the comparative study of tubercle bacilli will lead to some definite understanding on certain important questions, and eventually to more light on the whole subject of tuberculosis from the preventive as well as the therapeutic side.

The main questions proposing themselves to the investigator are:

1. The study of tubercle bacilli from different types of tuberculosis to determine their relation to the sputum bacillus and the bovine bacillus as regards virulence.

2. The study of the bacilli in primary intestinal disease and in all tubercular disease in children in which the source of infection is assumed to be outside of the family and possibly in the milk.

In attacking these problems the investigator should either keep strictly to the methods suggested in this article, if he wishes to use its results as a basis, or else, in all cases in which the bovine bacillus or one of abnormal virulence is suspected, he should study fresh cultures of the sputum bacillus side by side with the other.

THE RELATION OF THE TOXICITY OF DIPHTHERIA TOXIN TO ITS NEUTRALIZING VALUE UPON ANTITOXIN AT DIFFERENT STAGES IN THE GROWTH OF CULTURE.

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Until within a fairly recent time the filtered or sterilized bouillon in which the diphtheria bacillus had grown and produced its "toxin" was supposed to require for its neutralization an amount of antitoxin directly proportional to its toxicity as tested in guinea-pigs. Thus, if from one bouillon culture 10 fatal doses of "toxin" were required to neutralize a certain quantity of antitoxin, it was believed that 10 fatal doses from every culture, without regard to the way in which it had been produced or preserved, would also neutralize the same amount of antitoxin. Upon this belief was founded the Behring-Ehrlich definition of an antitoxin unit.

The results of tests by different experimenters with the same antitoxic serum but with different toxins proved this opinion to be incorrect. Ehrlich * deserves the credit for first clearly perceiving and publishing this. He obtained from various sources twelve toxins and compared their neutralizing value upon antitoxin; these tests gave most interesting and important information. The results in six toxins, which are representative of the twelve, are as follows: (See Table I.)

From the facts set forth in the tables Ehrlich has derived interesting theories, which, if true, would add greatly to our knowledge of toxins and would also have a very direct influence upon the present methods of standardizing antitoxin. He believes that the diphtheria bacilli in

* Ehrlich, Die Wertbemessung des Diphtherieheilserums und deren theoretische Grundlagen, *Klinisches Jahrbuch*, vi (1897).

TABLE I.—(From Ehrlich.)

Toxin specimen number of Ehrlich.	Estimated "minimal" fatal dose for 250-grm. guinea-pigs.	Smallest number of fatal doses of toxic bouillon required to kill a 250-grm guinea-pig within 5 days when mixed with 1 antitoxin unit. "L_+" Ehrlich.	Fatal doses required to "completely neutralize 1 antitoxin unit" as determined by the health of the guinea-pig remaining unaffected. "L_0" Ehrlich.	$L_+ - L_0$	Data upon "toxin" specimen given by Ehrlich.
2.	0.03	42	32	10	*Preserved 2 years.
4.	0.009	39.4	33.4	6	Old, deteriorated from .003 to .009.
7	0.0165	76.3	54.4	22	Fresh toxin, preserved with trikresol.
9.	0.039	123	108	15	A number of fresh cultures grown at 37° C. 4 and 8 days.
10.	0.001	29.2	27.5	1.7	Precipitated from greatly deteriorated "toxin."
12.	0.0025	100	50	50	Tested immediately after its withdrawal.

their growth produce toxin which, so long as it remains chemically unaltered, has a definite poisonous strength with a definite value in neutralizing antitoxin. This neutralization he believes to be a chemical union, in which 200 fatal doses of toxin for a 250-gramme guinea-pig combine with one unit of antitoxin. The toxin is, however, an unstable compound and begins to change almost immediately into substances which are not, at least acutely, poisonous, but which retain their full power to neutralize antitoxin. These substances, according to Ehrlich, fall into three groups. The first has more affinity for combining with the antitoxin than the toxin itself (protoxoids). The second has the same affinity (syntoxoids). The third has less affinity (epitoxoids).

*** For number 9 only does Ehrlich give the duration of the culture growth in the incubator by which the toxin was produced.**

According to him, if a mixture of toxoids and toxin is added to antitoxin, the protoxoids first combine with the antitoxin, then the syntoxoids and the toxin combine in equal proportions, so long as the supply lasts, with the amount of antitoxin remaining, or if there is a surplus, with enough to satisfy them; finally, if any antitoxin remains, the epitoxoids unite with it.

If to a mixture, in which all three toxoids as well as toxin have united with antitoxin, some additional toxic culture bouillon be added, the new protoxoids displace first the epitoxoids and then, if free protoxoids remain, the toxin and the syntoxoids from their antitoxin, and thus liberate as well as add free toxin to the solution.

Ehrlich states as an important law that toxin molecules change into toxoid molecules either by three toxin molecules becoming two toxoid and one toxin molecules, or that two toxin molecules change into one toxoid and one toxin molecule.

The practical conclusion which he draws is that from the absolute amount of poison in a given quantity of bouillon alone, nothing can be determined as to its neutralizing value for antitoxin, and that, therefore, the old Behring-Ehrlich method must be greatly modified; namely, that in any bouillon we must detect not only the amount of toxin but also of toxoids before we can judge of its neutralizing value. This he believes he can accurately accomplish.

He gives an interesting theory to explain the production of antitoxin in the blood. This he does upon the supposition that, when absorbed, the toxin combines with a portion of certain selected cells, and that this portion by its union with toxin becomes, at least physiologically, dead. The cell replaces this dead matter with new and similar substance; after several repeated losses and replacements of this substance the cells produce it in excess. This substance, whether originally in the normal cell or reproduced there, and whether remaining in the cell or thrown out into the circulation, is antitoxin.

Ehrlich attempts to separate into its constituent toxoids and toxin each culture bouillon by a formula worked out by him. He believes that his investigations show that the total amount of pure toxin which neutralizes one unit of antitoxin is 200 minimal fatal doses, and that in ordinary culture fluids, which are mixtures of toxoids and toxin,

an antitoxin unit neutralizes 200 fatal doses of toxin minus the number of toxin molecules changed into toxoid molecules. Letting A = number of toxin molecules and Z = epitoxoids, then one unit of antitoxin fully neutralized equals (200 — A — Z) toxoid-antitoxin + A toxin-antitoxin + Z epitoxoid-antitoxin. By toxoids are designated both protoxoids and syntoxoids.

To find epitoxoids his first step is to add as much toxin-toxoid mixture to the quantity of the toxic fluid which just neutralized one unit of antitoxin, as shown by the complete health after its injection of the guinea-pig, as will suffice to produce death on the fourth day. This amount will always be found to be above three fatal doses. This apparently excessive amount he believes to be explained by the fact that part of the almost harmless epitoxoids has been displaced by toxin. Ehrlich considers death on the fourth day to be caused by one free minimal fatal dose of toxin.

Let us, as Ehrlich, lump syntoxoids and protoxoids together under the name "toxoids," then suppose we have a toxin composed of 50 toxin molecules + 50 (syntoxoids and protoxoids) toxoids and 100 epitoxoids. To satisfy these 200 molecules of toxin, toxoids and epitoxoids we add one unit of antitoxin. After chemical combination we have 50 toxin-antitoxin and 50 toxoid-antitoxin and 100 epitoxoid-antitoxin. To displace the epitoxoids from the antitoxin we would have to add enough fatal doses to contain 100 molecules of toxin and toxoids, this would be then 50 of toxin and 50 of toxoid. A half molecule more of each would leave a pure toxin molecule, which would, according to Ehrlich, cause the guinea-pig's death in four days.

Ehrlich's formula to find the epitoxoids is:

$$\text{Epitoxoids} = \frac{200^{*}\ (L_{+}\dagger - L_{0}\ddagger - 1\S)}{L_{0} + (L_{+} - L_{0} - 1\S)} \quad \text{which with the above toxin would be} = \frac{200 \times 50}{50 + 50} = 100.$$

* 200 = number of molecules of toxin or toxoid required according to Ehrlich to neutralize one unit of antitoxin.

† L_{+} = fatal doses of toxin required to kill a guinea-pig in four days after having been mixed with one unit of antitoxin.

‡ L_{0} = fatal doses of toxin required to fully neutralize one unit of antitoxin.

§ 1, though given by Ehrlich, appears to us to be incorrect, for by his own statement one molecule of toxin and toxoid would be added, not 1 of toxin (1 fatal dose) alone. The amount to be subtracted from the difference between L_{+} and L_{0} would therefore be, not one, but a fraction of one.

The above summary merely gives an outline of Ehrlich's most interesting article. To become fully acquainted with the reasons for his theories the article itself must be carefully read. He concludes with the statement that his investigations have cleared up the difficulties surrounding the estimation of the amount of antitoxin in any given quantity of serum, and that the formula resulting from his investigations enables us to determine in any selected toxic diphtheria culture fluid the amounts of toxin and toxoid present, so that a unit of antitoxin is no longer a varying quantity, but of an exact and determinable size and one which can at any time be reproduced.

Interest in both his theoretical reasoning and in his practical conclusions led us to subject both to a series of tests which have, we believe, added some interesting facts to those already published by Ehrlich as well as cast doubts on some of his conclusions.

ORIGINAL INVESTIGATIONS.

In the following pages are given the results obtained by testing the toxicity in guinea-pigs and the neutralizing value upon antitoxin of fluid removed from bouillon cultures of different diphtheria bacilli after varying periods of growth.

Five cultures of diphtheria bacilli were used. Two of these were employed for the most important of the experiments and a short description of these may be of value.

One of them, No. 8, was obtained three years ago from a case of mild tonsillar diphtheria. For these three years this special culture has never been passed through animals, but has been transplanted to fresh bouillon every two to three days. It has kept almost intact its original virulence. It kills 250-grm. guinea-pigs in doses of about .005 cc. of a 48-hour bouillon culture in from four to five days, and produces a toxin in from two to five days of such a strength that usually .005 cc. of the filtered bouillon culture kills within five days most guinea-pigs weighing 250 grammes. The toxin produced by this bacillus has been constantly employed, with apparently uniform results in testing antitoxin.

The other culture, "Greenwood," was obtained six months ago from a case of diphtheria occurring in a localized epidemic in a town situated in an isolated mountainous district. This epidemic was remarkable for the

frequency with which paralysis appeared in rather mild cases. This peculiarity is noticed in its effects in animals. The other three cultures were obtained one year ago from cases of diphtheria occurring in New York City. These four cultures are of only moderate virulence and have been transplanted to fresh bouillon every few weeks.

The bouillon used for the development of toxin for these experiments, after being prepared from meat in the usual way, was first poured into a single vessel and made neutral to litmus. After adding to each litre of this common stock of neutral bouillon seven cubic centimetres of normal soda solution, the whole was boiled, filtered, poured into 600 ccm. flasks, stoppered with cotton and covered with filter-paper caps. The bouillon contained two per cent of Witte's peptone and filled the flasks one-half full. The bouillon, after thorough sterilization in the flasks, was inoculated from the pellicle of cultures made 48 hours previously. The growth of all cultures was abundant. After the period of growth at 35° C. had lasted the desired time, a measured quantity was withdrawn from each flask by means of sterilized pipettes. The amounts of fluid withdrawn were mingled together and, after the addition of 0.5 per cent of carbolic acid, stored in small sterilized glass bottles, stoppered and placed in the ice chest and there kept until used. As a rule a fresh vial of "toxin" was used for each experiment, as the exposure to the air of the toxin left in the bottle is apt to cause a deterioration in its strength.

The following tables give the actual and comparative toxicity and neutralizing value of "toxin" removed from bouillon cultures of diphtheria bacilli between the 6th hour and 49th day of their growth. Each table gives the details of a single experiment. It was found impossible to rely upon the L_+ and L_0 values unless all the guinea-pigs were inoculated at the same time. Otherwise slight changes in the condition of the animals and of the toxin were found to make the results somewhat inaccurate or at least subject to suspicion.

TABLE II.

GIVING RESULTS OF TEST OF TOXIN PRODUCED BY BACILLUS 8 AT END OF 6 HOURS' GROWTH IN SLIGHTLY ALKALINE 2% PEPTONE BOUILLON.

Toxin tested with one-tenth of an antitoxin unit on account of the slight toxicity of the fluid.

	Weight of guinea-pig.	Amount of toxic bouillon in cc.	Amount of antitoxic serum in cc.	Result in guinea-pig.	
1	278	3.00	*.00012	No deleterious effects.	
2	234	3.20	.00012	No deleterious effects.	
3	237	3.40	.00012	Slight temporary decrease in weight, no induration.	
4	245	3.60	.00012	Slight temporary decrease in weight, no induration.	
5	242	3.80	.00012	Slight temporary decrease in weight, slight induration.	
6	250	4.00	.00012	Considerable induration. Death 8th day.	
7	245	4.40	.00012	Died 6½ days,	
8	248	.3769	†.38	Died 7½ days,	Control tests of toxin to establish the minimal fatal dose for 250-gramme guinea-pigs.
9	265	.3816	.36	Died 6½ days,	
10	249	.44	.44	Died 5½ days,	
11	221	.40	.45	Died 4½ days,	
12	230	.45	.487	Died 3½ days,	
13	240	.5	.51	Died 3½ days,	

The minimal surely fatal dose for every 250-grm. guinea-pig, estimated from results of above tests, equals 0.45 cc. One-tenth of a unit, 0.00012 cc., of serum neutralized 3.40 cc., or 7.55 fatal doses, of toxin sufficiently to protect the health of the guinea-pig.

The "minimal fatal dose" is here defined, so as to be in accord with Ehrlich, as the amount which will kill most guinea-pigs of 250 grms. weight on the fourth day and the remainder that live longer certainly on the fifth day. The amount of toxin given is calculated according to the gramme weight of the pig, since experience has taught that on the average guinea-pigs weighing between 225 and 275 grammes are susceptible to toxin directly proportional to their weight.

*** .00012 cc. of this serum equals one-tenth of an antitoxin unit as standardized by Ehrlich's test serum.**

† Equivalent amount of toxin for a 250-grm. guinea-pig.

TABLE III.

GIVING RESULTS OF TEST OF TOXIN PRODUCED BY BACILLUS 8 AT END OF 22 HOURS' GROWTH IN SAME CULTURE AS TESTED AT 6 HOURS.

	Weight of guinea-pig.	Amount toxic bouillon in cc.	Amount serum in cc.	Result in animal.		
				Weight on 5th day.	Amount induration on 5th day.	General condition on 5th day, if alive.
1	260	2.00	.0012	265	None.	Good.
2	257	2.10	.0012	242	Slight.	Good.
3	250	2.16	.0012	237	Considerable.	Good.
4	252	2.20	.0012	215	Considerable.	Sick.
5	265	2.36	.0012	225	Considerable.	Dying.
6	252	2.38	.0012		Marked.	Dead on 5th day.
7	250	2.40	.0012			Dead on 3rd day.
8	248	.016	*.016	Died 6th day.	Control tests of toxin to establish the minimal fatal dose for a 250-grm. guinea-pig.	
9	248	.018	.018	Died 4th day.		
10	232	.0168	.018	Died 6th day.		
11	238	.020	.021	Died 3rd day.		

Minimal fatal dose estimated to be about .019 cc.
$L_0 = 2.06 = 108.4$ minimal fatal doses.
$L_+ = 2.39 = 125.8$ minimal fatal doses.
$L_+ - L_0 = .33 = 17.4$ fatal doses.

Epitoxoids by Ehrlich's formula $\frac{200 \times 16.4}{108.4 + 16.4} = 27.8$.

.0012 cc. of this serum equals one unit of Ehrlich's test serum.

TABLE IV.

GIVING RESULTS OF TEST OF "TOXIN" PRODUCED BY BACILLUS 8 AT END OF 48 HOURS' GROWTH. TESTED VII-6-'98.

	Weight of guinea-pig.	Amount of toxin in cc.	Amount serum in cc.	Result in animal.		
				Weight on 5th day.	Amount of induration on 5th day.	General condition on 5th day.
1	220	.500	.0012	239	Pin-point.	Good.
2	224	.512	.0012	222	Bean-sized.	Good.
3	288	.524	.0012	280	Slight.	Good.
4	257	.536	.0012	248	Moderate.	Good.
5	270	.548	.0012	250	Considerable.	Good.
6	262	.560	.0012	238	Marked.	Good.
7	235	.582	.0012	176	Marked.	Sick (died vii–13).
8	265	.594	.0012	217	Marked.	Sick (died vii–14).
9	250	.604	.0012	192	Marked.	Very sick (died vii–12).
10	280	.616	.0012			Died vii–10 (4th day).
11	264	.628	.0012			Died vii–9 (3rd day).
12	260	.0062	†.0060	Died vii–12 (6th day).		Control tests of toxin to establish the four-day or "minimal fatal" dose for 250-grm. guinea-pig.
13	259	.0067	.0065	Died vii–12 (6th day).		
14	272	.0076	.0070	Died vii–12 (6th day).		
15	246	.0073	.0075	Died vii–10 (4th day).		
16	246	.0083	.0085	Died vii–12 (6th day).		
17	248	.0089	.0090	Died vii–9 (3rd day).		

.0075 estimated minimal fatal dose for 250-grm. guinea-pig.
$L_+ = .610 = 81.33$ fatal doses.
$L_0 = .512 = 68.26$ fatal doses.
$L_+ - L_0 = .098 = 13.07$ fatal doses.
Epitoxoids by Ehrlich's formula 30.'

* Equivalent of toxin dose for a 250-grm. guinea-pig.
† Equivalent amount for a 250-grm. guinea-pig.

TABLE V.

GIVING RESULTS OF TEST OF TOXIN PRODUCED BY BACILLUS 8 AT END OF 6 DAYS.

Weight of guinea-pig.	Amount of toxin in cc.	Amount of antitoxin in cc.	Result in animal.	
200	.48	.0012	Remained full weight and alive.	
210	.48	.0012	Died with paralysis after 20 days; on 11th well and full weight.	
214	.51	.0012	Died in 3½ days.	
217	.53	.0012	Died in 4½ days.	
212	.0047	*.0055	Died 4th day.	Control tests to establish four-day fatal dose for guinea-pigs.
254	.0056	.0055	Died 7th day.	
208	.0050	.0058	Died 7th day.	
208	.0052	.0062	Died 4th day.	

Estimated minimal fatal dose, .0062.

L_+=.535=86.3 fatal doses.

L_0=.475=76.6 fatal doses.

$L_+ - L_0$=.06=9.7 fatal doses.

Epitoxoids by Ehrlich's formula=20.16.

The toxin from the Greenwood culture could not be tested before 3 days, since up to 48 hours the culture fluid was not toxic. Minimal fatal dose 48 hours = 5 cc.

TABLE VI.

SHOWING RESULTS OF TESTING TOXIN REMOVED AT END OF 72 HOURS' GROWTH FROM GREENWOOD CULTURE.

Weight of guinea-pig.	Amount of toxin in cc.	Amount of serum in cc.	Result in animal.	
220	5.90	.0012	Unaffected.	
260	6.00	.0012	Slight induration.	
248	6.80	.0012	Died 7th day.	
225	7.30	.0012	Died 2nd day.	
228	.050	†.055	Died in 6 days.	Control tests of toxin for minimal fatal dose.
238	.056	.060	Died in 3½ days.	
210	.0546	.065	Died in 3½ days.	

Four-day or minimal fatal dose of toxin estimated at .058.

L_0=about 5.95=102.6 fatal doses.

L_+=about 7.00=120.7 fatal doses.

$L_+ - L_0$=about 1.10=18.1 fatal doses.

Epitoxoids by Ehrlich's formula=29.

* This culture was developed in a different flask of bouillon from that used for the other tests. The bouillon was from different meat, but was otherwise similar.

† Equivalent for 250-grm. guinea-pig.

TABLE VII.

SHOWING RESULTS OF TESTING TOXIN REMOVED AT END OF 6 DAYS' GROWTH FROM GREENWOOD CULTURE.

Weight of guinea-pig.	Amount of toxin in cc.	Amount of serum in cc.	Result in animal.	
229	.4172	.00012	On 2nd day lost 13 grms., but on 4th day regained 7 grms. and remained well.	
193	.4323	.00012	Died in 50 hours.	
195	.0312	.00400	Died in 2½ days.	Control tests for minimal fatal dose of toxin for 250-grm. guinea-pig.
209	.038	.00452	Died in 3½ days.	

Estimated minimal fatal dose about .038.

One-tenth unit .00012 completely protected guinea-pig from .4172 cc. of toxin = 10.9 fatal doses.

L_+ would equal about 104 fatal doses.

TABLE VIII.

SHOWING RESULTS OF TESTING TOXIN REMOVED FROM SAME FLASK OF GREENWOOD CULTURE AT END OF 7 WEEKS.

Weight of guinea-pig.	Amount of toxin in cc.	Amount of serum in cc.	Result.	
220	2	.0012	Unaffected.	
250	3	.0012	Slight induration, temporary loss in weight.	
217	4	.0012	Temporary induration and loss in weight. Paralysis 20th day. Death 25th day.	
210	5	.0012	Death on 5th day.	
215	6	.0012	Death on 2nd day.	
245	.10	*.1020	Lived, loss 30 grams in weight.	Control tests of toxin to establish the four day "minimal" fatal dose for a 250-grm. guinea-pig.
270	.12	.1110	Lived.	
237	.13	.1390	Lived after recovering from paralysis.	
234	.14	.1495	Died 22nd day, paralysis.	
227	.16	.1760	Died 24th day, paralysis.	
250	.18	.1800	Lived after recovering from paralysis.	
262	.20	.1907	Died 2nd day.	
234	.19	.2030	Died 3rd day.	

Minimal or four-day fatal dose estimated from above tests at .19 cc.

L_+ = about 5.10 = 26.8 fatal doses.

L_0 = " 3.00 = 15.8 " "

$L_+ - L_0$ = about 2.10 = 11.0 fatal doses.

Epitoxoids by Ehrlich's formula, $\frac{200 \times 10}{15.8 + 10} = \frac{2000}{25.8} = 77.5$.

The unusual amount of paralysis in the guinea-pigs receiving much less than the four-day fatal dose is very striking in the above series. Toxin from this culture of shorter duration of growth had this same characteristic, but to a less extent.

* This column of figures shows equivalent toxin for a 250-grm. guinea-pig.

TABLE IX.

Giving Minimal Fatal Dose; Number of Fatal Doses of Toxin Neutralized by One Unit of Antitoxin Sufficiently to Protect Life of Animal for 4 Days, L_+; to Protect the Health of the Animal, L_0; the Difference between L_+ and L_0; and the Amount of Epitoxoids as determined by Ehrlich's Formula.

Culture 8 development.	Minimal fatal dose in cc.	L_+ in minimal fatal doses.	L_0 in minimal fatal doses.	$L_+ - L_0$ in minimal fatal doses.	Epitoxoids (Ehrlich). Number of molecules in amount of "toxin" required to neutralize one unit.
6 hours	.45	*72			
22 hours	.019	125.8	108.4	17.4	27.8
‡28 hours	.018	91.6	77.7	13.9	28
48 hours	.0075	81.33	68.26	13.07	30
†48 hours	.016	81.2	73.4	7.8	17
† 6 days	.0062	86.3	76.6	9.7	20.16
‡ 3 weeks	.0095	42.3	34.2	8.1	34.3
‡ 8 weeks	.038	31.6	21	10.6	46.8
Culture Greenwood.					
3 days	.058	120.7	102.6	18.1	29
6 days	.038	*104			
7 weeks	.19	26.8	15.8	11	77.5

The above tabulated results obtained from testing the absolute and comparative toxicity and neutralizing value upon antitoxin of fluid removed at different periods in the growth of two cultures are certainly striking, and fully substantiate Ehrlich's claim that the original Behring-Ehrlich definition of an antitoxin unit is very insufficient. The small number of fatal doses of 6-hour "toxin" which .1 unit of

* Estimated from the number of fatal doses sufficiently neutralized by one-tenth of a unit to protect the health of the guinea-pig.

† The bouillon in which this toxin was formed was different from that in which the others developed.

‡ Tests completed too late to insert tables. Details carried out as in those where full tables have been given.

antitoxin neutralized—7.5, which is the equivalent of about 72 for one unit—surprised us, for, according to Ehrlich's theories, we should expect the "toxin" to be nearer purity then than at 22 hours. It is probable that changes produced in the culture bouillon account for this and later unexpected results. A second peculiar result of the test was the rapid decrease (from 126 to 91) in the number of fatal doses required to neutralize one unit between the 22nd and the 28th hour, to be followed by slight irregular changes (81 at 48 hours, 86 at 6 days) before the final apparently permanent tendency to decrease (42 at 3 weeks, 31 at 6 weeks).

"Toxin" from culture Greenwood in its test at 3, 6 and 49 days shows also very marked variations in the actual and in the comparative toxicity and neutralizing strength of the culture bouillon. We note that at 3 days the culture fluid required 120 fatal doses to neutralize one unit, about the same amount as culture 8 required at 22 hours. The variation in these two cultures is more apparent than real in that, although the neutralizing value of a fatal dose was different at similar periods of growth, nevertheless when we consider that culture 8 began to produce toxin immediately and culture Greenwood only at the end of 48 hours, we see that at about the same period in their toxin production the neutralizing value of a minimal fatal dose was about the same in both.

Before discussing further these tests, let us examine the neutralizing value of a fatal dose in different cultures, grown in similar bouillon, at the same period of growth and of the same culture in bouillon from different sources.

TABLE X.

THE ACTUAL AND COMPARATIVE TOXICITY AND NEUTRALIZING VALUE OF THE FLUID FROM FIVE DIFFERENT CULTURES AFTER SIX DAYS OF GROWTH.

Culture.	Minimal fatal dose for 250-grm. guinea-pig.	Number of fatal doses neutralized by one-tenth of an antitoxin unit.
8	.006	9
213	.085	9.76
236	.030	13.9
238	.043	9.07
Greenwood	.038	10.9

The results given above from the tests of the neutralizing value of the toxin removed from five cultures after 6 days' growth at 36° C.,

the time when toxin is usually withdrawn by us, reveals a great lack in uniformity in the neutralizing value of a fatal dose, although no such difference exists as in the case of the same culture fluid at widely different periods of development.

The actual and comparative toxicity and neutralizing value of the "toxin" produced by the same bacillus in the fluid removed from two flasks of bouillon derived from different meats but of equal alkalinity is shown by comparison of Tables IV and XI.

The data upon the tests of one culture bouillon removed 48 hours after inoculation with bacillus 8 have been given already in Table IV.

The minimal fatal dose was .0075.

$L_+ = .610$ or 81.33 fatal doses.

$L_0 = .512$ or 68.26 fatal doses.

The data of the test of the "toxin" developed by the same bacillus 8 in a different bouillon is given fully in Table XI, since a comparison of the two is important, in that the uniformity of the two toxins indicates the possibility of the same bacillus under suitable conditions furnishing a toxin of always about the same relative toxicity and neutralizing power.

TABLE XI.

TEST OF TOXIN PRODUCED BY BACILLUS 8 AT THE END OF 48 HOURS IN A BOUILLON DIFFERENT FROM THAT RECORDED IN TABLE IV.

	Weight of guinea-pig.	Amount of toxic bouillon in cc.	Amount of serum in cc.	Result in animal.	
1.	265	1.125	.0012	Unaffected.	
2.	263	1.15	.0012	Slight skin necrosis. No loss in weight.	
3.	270	1.175	.0012	Slight induration. No loss in weight.	
4.	277	1.20	.0012	Death 7th day.	
5.	257	1.30	.0012	Death 4th day.	
6.	250	.011	*.011	Died 6th day.	Control tests of toxin to establish four-day fatal dose in guinea-pigs.
7.	235	.0112	.012	Died 21st day after paralysis.	
8.	250	.0106	.013	Died 6th day.	
9.	205	.0118	.014	Died 6th day.	
10.	264	.0158	.015	Died 5th day.	
11.	240	.0153	.016	Died 4th day.	

Minimal or four-day fatal dose estimated at .016 cc.
$L_+ = 1.30 = 81.2$.
$L_0 = 1.175 = 73.4$.
$L_+ - L_0 = 125 = 7.8$.
Epitoxoids "Ehrlich" = 17.

* The equivalent in a 250-grm. guinea-pig of the amount of toxin given in the actual animal.

Certainly the toxins produced by bacillus 8 in these two different bouillons as given in the Tables IV and XI are remarkably similar in the neutralizing value of a fatal dose, although the toxicity of one toxin as shown in Table XI is only one-half of the other as shown in Table IV. Six toxins developed during the six-days' growth of cultures of No. 8 in slightly alkaline bouillon have at different times in the past three years been used in antitoxin tests, and all have had nearly the same antitoxic neutralizing value for a fatal dose.

These results have encouraged us to hope that, with a closer study of the relations existing between the changes taking place in the bouillon during the different stages of the growth of the bacilli, and of both the development of the toxin and its neutralizing value, we may be able to obtain from any culture of known characteristics at a certain period of its toxin production a toxin of always about the same neutralizing power. A comparison between different laboratories of the neutralizing value of the toxins produced by their bacilli would help to obtain this information.

Later it would be a very simple matter to select one of the cultures which proved most constant in its characteristics and distribute this to all the testing laboratories of a country. In this way the relative toxicity and neutralizing value of the culture fluid grown under the most varying conditions would soon become known.

When we examine the results of the above tests they do not appear to us to reveal the presence of a substance of such a nature as that of the epitoxoids, as defined by Ehrlich, nor do they seem to indicate the possibility of detecting, except in so far as comparative tests suggest, the purity or constituents of a fatal dose of diphtheria culture fluid or "toxin." If epitoxoids have the properties described by Ehrlich, then they should in proportion to their presence increase the gap between L_+ and L_0, but we find no evidence of this in the tables for the fluids having by Ehrlich's formula and theory far the greatest amount of epitoxoids; the deteriorated seven and eight-weeks' toxins have almost the smallest gap between L_+ and L_0. Again we find decidedly more epitoxoids at the end of 22 and 48 hours' growth of the culture 8 than at 6 days and 3 weeks. This certainly seems unlikely,

for with the increased amount of deteriorated toxin present in the culture of longer growth in the incubator, we should expect an increase, not a decrease of epitoxoids, if such exist. Again the remarkable variations in number of fatal doses required to neutralize one unit between the 6, 22, 28 and 48-hour toxin are difficult to understand on Ehrlich's theory.

There is indeed, with the use of increased quantities of antitoxin, a larger and larger gap between the amount of toxin which just suffices to produce a slight temporary induration or effect upon the health of the test guinea-pig and the amount which kills upon the fourth day. This difference is about three fatal doses when one-tenth of a unit is neutralized and about eight to fourteen when one unit is employed. In the above tables it will be seen that there is an average of about 8 fatal doses between the amount of toxin which kills a guinea-pig on the fourth day and the amount which kills at a later period from paralysis or increased weakness. This amount is not greater, however, in the old deteriorated toxin than in the fresh, and shows great variations.

This inhibition, through antitoxin, of the otherwise quickly fatal effect of such an excess as six to ten fatal doses of toxin, suggests a difficulty in accepting as proven the chemical theory of the neutralizing effect of antitoxin as being due to its direct combination with the toxin.

Ehrlich not only forms a theory to explain the varying neutralizing value of a fatal dose of toxin, but through a formula, as already stated in the earlier part of this article, he attempts, and, as he believes, attempts successfully, to separate a fatal dose of " toxin " into its constituents and thus detect with accuracy what amount of antitoxin it should neutralize. He claims that by such separation only can a toxin and therefore an antitoxin be properly standardized. Neither his theoretical reasoning nor the results of the working of his formula seem to us convincing of the accuracy of these claims. Not only the weight of Ehrlich's authority, but the practical importance to all those who test antitoxin of his conclusions, demand that we discuss his views.

Upon the four-day limit of life for a guinea-pig which has received a fatal dose of toxin he obtains the values of L_+, L_0, and therefrom the epitoxoids. A dose of toxin which kills most 250-grm. guinea-pigs in four days is for practical purposes called the minimal surely fatal dose for all pigs of that weight. It is, however, an arbitrary standard, and is in fact much more than the minimal fatal dose for most guinea-pigs. A glance at the control pigs in any table—Table IV is a good example—will show how much beyond the actual fatal dose for guinea-pigs is the amount which kills on the fourth day. A minimal fatal dose for a single pig will kill at the end of a week or ten days, or later still by paralysis in the 2nd or 3rd week. This seems so apparent as hardly to need stating.

If, however, Ehrlich should choose such a real limit, or even put it at the sixth or seventh day, it would entirely alter the values of L_+ and L_0, and therefore the results of his formula. The value of L_+ can be fairly accurately determined, but not that of L_0, since slight induration or loss of weight may or may not occur in different animals from the same amounts. His assumption that one unit of antitoxin neutralizes 200 fatal doses of pure toxin or of its derivatives seems to us purely theoretical. Even if the toxin molecules be divided as he believes, still in any given bouillon there is actual destruction, as well as production, going on all the time of all substances, not only those toxic to guinea-pigs, but also of those which, though not toxic, still neutralize antitoxin.

If Ehrlich's formula really revealed the nature of a "toxin," it should answer when employed with larger or smaller amounts of antitoxin than one unit. The L_+, L_0, and the minimal fatal dose should have the same relative value when .1, 1 or 10 units of antitoxin are mixed with varying amounts of toxin. The 200 would merely become 20 or 2000. If, however, we test the formula by solving the same toxin when different known quantities of antitoxin are employed, the amount of epitoxoids will vary with every test; thus toxin from culture Greenwood of three days' growth tested against 1 unit shows:

TABLE XII.

GREENWOOD TOXIN OF THREE DAYS' GROWTH, THE FLUID BEING THE SAME AS TESTED IN TABLE VI AND TESTED AT THE SAME TIME.

	Weight of guinea-pig.	Amount of toxic bouillon in cc.	Amount of serum in cc.	Result in animal.
1.	245	.80	.00012 = .1 unit	Unaffected.
2.	247	.84	.00012 = .1 unit	Died 8th day.
3.	222	.86	.00012 = .1 unit	Died 8th day.
4.	247	.88	.00012 = .1 unit	Died 4th day.

Minimal fatal dose .058 (see Table VI).

$L_+ = .88 = 15.2$ fatal doses.

$L_0 = .80+ = 13.9$ fatal doses.

$L_+ - L_0 = .08+ = 1.3$ fatal doses.

Epitoxoids in .1 unit $= \frac{20 \times (L_+ - L_0 - 1)}{13.9 + (L_+ - L_0 - 1)} = \frac{20 \times .3}{13.9 \text{ plus } .3} = .42$

Epitoxoids in 1 unit = 4.2.

Epitoxoids in the same toxin tested by formula when 1 unit of antitoxin is employed = 29.

This table illustrates also that the new method of grading the value on the L_+ amount with one unit is somewhat more severe than the old method of grading upon the L_0 with one-tenth of an antitoxin unit.

SUMMARY AND CONCLUSIONS.

Until recently diphtheria "toxin" was supposed to be a single definite substance and to have a definite toxicity in animals and neutralizing power for antitoxin. A fatal dose of toxin, without regard to the conditions under which it was produced or preserved, was supposed to require always the same quantity of antitoxin to neutralize it. Ehrlich's researches have completely done away with this theory, and have substituted for it one which assumes the toxin to be only at its origin a single definite chemical compound with definite physiological and antitoxic properties. According to Ehrlich the toxin is an unstable substance which readily loses its toxicity, while at the same time its affinity for antitoxin may be either increased or decreased. Its neutralization by antitoxin he considers to be due to a chemical union between the toxin and the antitoxin.

The results of our experiments as detailed in this paper are fully in accord with those published by Ehrlich, as to the varying neutralizing

value of a minimal fatal dose of "toxin"; they, however, go further and indicate roughly a general law in accordance with which these changes occur.

The neutralizing value of a fatal dose of toxin is at its lowest in the culture fluid when the first considerable amounts of toxin have been produced. After a short period, during which the quantity of toxin in the fluid is increasing, the neutralizing value of the fatal dose begins to increase, at first rapidly, then more slowly.

While the culture is still in vigorous growth and new toxin is being produced, the neutralizing value of the fatal dose fluctuates somewhat, but with a generally upward tendency. After the cessation of toxin production the neutralizing value of the fatal dose increases steadily until it becomes five to ten times its original amount.

In our experiments the greatest value for L_+ was 126, the least 27. As at 6 hours L_+ was only 72 and at 28 hours only 91, we doubt whether L_+ ever reaches above 150, and therefore hardly expect Ehrlich's figures of 200 to be realized. When we seek to analyze the above-described process, we find certain facts which seem partly to explain it. Experiments have shown that filtered toxin, preserved for any length of time in conditions under which access of air occurs, gradually loses in both its toxicity and neutralizing power, and that it loses more rapidly in the former property than in the later. Thus, while the fatal dose of a toxin preserved for one year rose from .01 cc. to .55 cc., it lost only half as much in neutralizing value, one unit neutralizing at first 1 cc., at the end of the year 25 cc. These processes take place more rapidly at room temperature than in the ice chest, and in the incubator than in the room.

In the fluid holding the living bacilli we have, therefore, after the first few hours of toxin formation, a double process going on, one of deterioration in the toxin already accumulated, which tends to increase the neutralizing value of the fatal dose, the other of new toxin formation, which probably tends to diminish the neutralizing value. The chemical changes produced by the growth of the bacilli in the bouillon tend to aid one or the other of these processes and so to make from hour to hour slight changes in the value of the fatal dose. Later,

with the period of cessation of toxin production, the gradual deterioration of the toxicity alone continues, and the fatal dose gradually and steadily increases in its neutralizing value.

Ehrlich's theories, as to the splitting up of "toxin" into toxoids having little or no toxicity but on the average full neutralizing power for antitoxin, have not in our opinion been substantiated by the results of these experiments. The difference between the amount of toxin mixed with a unit of antitoxin which causes the first symptoms and that causing death upon the fourth day would be, it is true, explained by his theory, but the failure of this difference to be greater where, by his theories, epitoxoids should be in great abundance prevents our acceptance of his views. The fact of the greater neutralization value of a fatal dose of a deteriorated toxin would be accounted for on his protoxoid theory. This, however, is not proof of its correctness, as other theories, such as the production by the diphtheria bacillus of two or more closely allied toxins, similar to the allied alkaloids produced by plants, would equally account for it, if we supposed the one which had the greater neutralization value was more resistant to destruction than the other. We only advance this theory to call attention to the fact that many theories can on paper explain a process without necessarily being thereby established.

Even if his theories prove partially correct, we feel certain that his formula for standardizing toxins is founded upon error and cannot be employed for the purpose intended by him.

While we do not believe, therefore, that he has changed the principles of testing antitoxin, yet we believe he has contributed greatly to uniformity in results by calling attention to the necessity of selecting a suitable toxin and by employing and distributing an antitoxin as a standard to test toxins by. In this way smaller testing stations can make their results correspond with those of the central station.

In spite of the great variations in the neutralizing value of a fatal dose in different toxins, we do not believe there has been any such great difference in the toxins used by the different stations for testing purposes. Most laboratories have taken the culture fluid at about the time of its greatest toxicity, and the neutralizing value of a fatal dose

of this toxin would seldom vary more than 10 per cent above or below the standard now adopted in Germany by the government testing station, this latter being presumably as close as possible to that used to establish the original Behring-Ehrlich unit.

Where error has been made, it has usually been by taking too old culture fluids, which would cause the antitoxin strength of samples tested to be estimated below and not above its real value. Culture 8, which is used not only by us but by many other laboratories in the United States and Europe, fortunately produces on the 6th day, the time at which the culture is usually removed, a toxin which grades Ehrlich's antitoxin within five per cent of the strength given by him.

We believe that by using such a bacillus, we can, after gaining a fuller knowledge of its characteristics, obtain a toxin of a known and suitable neutralizing value, and thus always correctly standardize an antitoxic serum. Meanwhile a fairly permanent antitoxin, such as Ehrlich provides, is of immense value in insuring a uniform though not necessarily correct standard among the different testing stations and in allowing of comparison between them.

AN EXPERIMENTAL STUDY OF THE DIRECT INOCULATION OF BACTERIA INTO THE SPLEEN OF LIVING ANIMALS; AND A CONTRIBUTION TO THE KNOWLEDGE OF THE IMPORTANCE OF A LESION IN ANIMAL TISSUE FOR THE LODGMENT AND MULTIPLICATION OF BACTERIA WITHIN IT.

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The methods of experimental infection which are at the present time mostly used are subcutaneous or intraperitoneal inoculation or intravenous injection of the micro-organisms employed. The final object to be attained is the introduction of the organisms into the circulation, and the different results obtained by these various methods of inoculation are largely due to a mechanical factor—to the rapidity with which the organisms and their products are absorbed from the locality in which the injection was made.

It occurred to the writers that new characteristics in the forms of infection might be obtained from a systematic study of inoculations made primarily into one of the internal organs.

It seems fair to suppose that specific metabolic processes take place in all organs having special functions, and it is not illogical to assume that the specific catabolic processes of an organ might have a characteristic effect upon bacteria, more particularly when they are unchanged by previous contact with the blood or lymph.

The spleen, above all the internal organs, seems to harbor the specific micro-organisms in certain of the infectious diseases, and for this reason, notwithstanding the fact that the exact function of the spleen seems to be little understood, this organ was selected for our experiments as the seat of direct infection. From its peculiar anatomical structure, some hold the opinion that the spleen acts as a filter

and holds back bacteria from the blood passing through it. It might also be claimed that the bactericidal power of the blood is increased in the spleen, for on entering the organ, the blood leaving the capillaries comes in close contact with the lymphoid tissue where leucocytes abound, and it is quite generally believed that a close connection exists between leucocytes and the bactericidal action of body fluids.

Now, on the other hand, H. Buchner* claims that wherever disintegration of the red blood corpuscles occurs, the alexines fail to display their full power; and according to the prevailing opinion the erythrocytes are broken down in large numbers in the spleen. Hence it might be assumed, as a fair hypothesis, that while the non-corpuscular alexines are easily carried from the spleen by the blood, that the corpuscular elements, including the micro-organisms, remain in the spongy tissue of the spleen, and the bacteria, well nourished by the broken down erythrocytes, resist the attacks of the remaining alexines.

Our first problem then was to determine the fate of bacteria when inoculated directly into the spleen.

The animals experimented on were chiefly rabbits; the inoculations were mostly of broth cultures of B. coli communis, and the results obtained were controlled by inoculations of broth cultures of B. typhosus and Staph. pyogenes aureus. The inoculations, made with a hypodermic syringe, were directly into the substance of the spleen, the needle being introduced into the ventral end, *i. e.* that end of the spleen lying nearest the abdominal wall. The operation for exposing the spleen was made under ether anesthesia, the incision in the median line of the abdomen causing little or no loss of blood. In none of our numerous experiments did a secondary infection at the site of operation occur, the wound in the abdominal wall healing *per primam.*

The slight bleeding from the spleen, caused by the puncture of the hypodermic needle, was arrested either by a ligature thrown around the needle or by the actual cautery.

In due time each animal was killed with chloroform and the autopsy

* *Centralbl. f. Bakt. u. Parasit.*, vi, No. 1.

performed immediately under strict aseptic precautions. After opening the thorax and abdomen the spleen was at once removed and placed in a sterilized Petri dish, and cultures were then made in 10 per cent nutrient gelatin, reacting 1.5 per cent acid to phenolphthalein, from the blood in the left ventricle, hepatic vein, liver and left kidney, as a routine procedure; other cultures being made from time to time as hereafter noted. Gelatin plates were then made from the spleen, which for convenience was divided into three parts; the ventral end, or that through which the inoculation was made, being designated I, the middle third, II, and the dorsal third, III. Three large "loops" of the juice of the spleen were used for making the plates and other plates were made with bits of tissue torn out from the substance of the organ. All plates were kept under observation for not less than seven days. Cover-glass smears were also made from the tissues plated.

*Experiment 1.** Medium-sized rabbit; 5 minims of 24 hours broth culture of B. coli communis injected into spleen. Autopsy after 40 hours. Plates from parts I, II and III of the spleen remained sterile.

Experiment 2. Small rabbit; 6 min. of 48 hours broth culture of B. coli comm. injected into spleen. Autopsy after 40 hours. Spleen plates; I showed 4 colonies; II, 2 colonies; III, 5 colonies. Plates from liver, 6 colonies; from hepatic vein, 2 colonies; and from left ventricle, 4 colonies.†

Experiment 3. Small rabbit; 6 min. of 24 hours broth culture of B. coli comm. injected into spleen. Autopsy after 14 hours. Spleen plates; I, 3 colonies; II, 7 colonies; III, 0. Plates from liver, 0; from kidney, 1 colony.

Experiment 4. Large rabbit; 10 min. of 24 hours broth culture of B. coli comm. injected into spleen. Autopsy after 14 hours. Spleen plates; I, 11 colonies; II, 43 colonies; III, 3 colonies. Plates from liver, 32 colonies; from hepatic vein, 15 colonies; from left ventricle, 133 colonies; and from left kidney, 0.

* The experiments are numbered in the order in which they are reported, not in the order in which they were made.

† The number of colonies reported are the average of two or more plates made from the same locality, the number occurring on each of the several plates not being far from the average obtained.

Experiment 5. Small rabbit; 5 min. of 20 hours broth culture of B. coli comm. injected into spleen. Autopsy after 3 hours and 20 minutes. Spleen plates; I, 0 colonies; II, 13 colonies; III, 3 colonies. Plates from liver, 2 colonies; from hepatic vein, 0; from left ventricle, 5 colonies; and from left kidney, 0.

Experiment 6. Small rabbit; 25 min. of 4 days broth culture of B. coli comm. injected into spleen. Rabbit died in 3½ days; autopsy six hours after death. Spleen plates; I, 200; II, 350; III, 150 colonies of B. coli communis; no other colonies present.

Experiment 7. Large rabbit; 25 min. of 48 hours broth culture of B. coli comm. injected into spleen. Died after 24 days. Plates from the three parts of the spleen, from the liver, hepatic vein, left ventricle and left kidney all remained sterile.

It seems unnecessary to mention further experiments of this kind; the few here quoted from our protocols show that large numbers of bacteria injected into the spleen soon disappear.

The organisms injected into the spleen were neither kept back mechanically, as in a filter, nor did they find within the spleen a soil favorable to their multiplication. Although the 5-minim dose of broth culture, which was injected, corresponds to about one-third of the entire volume of the spleen and contained many millions of bacteria, the plates made from the juices and tissue of the spleen were for the most part sterile, and even in the plates made only 3 hours and 20 minutes after the inoculation, but few colonies developed. Controls of the cultures employed for the inoculations were made in several instances by adding a small loopful of the culture to 5 cc. of sterile broth and immediately plating three loopfuls of this mixture in nutrient gelatin. The number of colonies developing on these plates varied from 3000 to 10,000.

These control experiments assured us that many millions of bacteria disappeared from the spleen within a short time.

The question now arose: Is this large number of bacteria rapidly destroyed in the spleen, or are these bacteria carried out from the spleen in immense numbers by the blood current?

The best method to exclude the latter proposition would have been to tie all the splenic veins, but this we could not do in the small animals

experimented with. In a single case an attempt was made to prevent the flow of blood from the spleen by tying the portal vein.

Experiment 8. Medium-sized rabbit; ligated portal vein and then injected 5 min. of broth culture of B. coli comm. into the spleen. Animal died in 30 minutes. The spleen was removed, placed in a sterilized Petri dish and plated 14 hours later. Juices and tissue were plated from five different incisions made into the spleen. All plates showed an innumerable growth of B. coli and no contaminations were found.

In the other experiments it seemed preferable to tie the splenic vessels themselves, but we were obliged to ligate the arteries together with the veins.

Experiment 9. Large rabbit; tied all splenic vessels and injected into the spleen 5 minims of broth culture of B. coli communis. Autopsy after 40 hours. Spleen plates I, II, III all show an innumerable growth of B. coli communis.

Here the bacilli could not migrate into the circulation, and indeed there was every evidence of an active growth of them within the spleen. We have to remember, however, that the supply of fresh blood and with it the supply of fresh alexines were cut off, while the amount of alexines present in the spleen at the moment of ligating the vessels was probably out of proportion to the number of bacteria introduced into the spleen. It is now generally accepted that the effect of the bactericidal elements in the blood is but little apparent in the presence of large numbers of bacteria, and that after the bactericidal powers are exhausted the surviving bacteria multiply with great rapidity.

Anatomists teach that the capillaries in the spleen resolve and the blood enters the tissue; and the assumption seems justified, and is indeed tacitly made, that quite a fair communication exists between the interstitial spaces of the spleen. We therefore resorted to the plan of ligating only a part of the vessels pertaining to the spleen, anticipating that the migration of bacteria from the ligatured portion would be considerably restricted, while there might be some, perhaps an abundant supply, of the bactericidal factors to this part provided by

the neighboring parts of the spleen through which the blood was allowed to flow.

Experiment 10. Small rabbit; tied about ⅓ of the vessels on the ventral end of spleen, and through the ventral (ligated) end injected 5 min. of a 24 hours broth culture of B. coli comm. into the middle portion of spleen. Tip of spleen tied. Autopsy after 40 hours. Tip of spleen was found detached from the rest of the organ and imbedded in the mesentery; the ventral third of the spleen, the vessels of which had been ligated, was considerably thickened and paler than the remaining part of the organ, and distinctly separated from it by a ridge or line of demarcation. The plates made from the ligatured part of the spleen (I) were overcrowded with colonies and the plates from parts II and III showed growth of colonies which were too abundant to count. Colonies were most abundant in plates from part I, distinctly less abundant in plates from part II, and still less numerous in the plates from part III.

Experiment 11. Medium-sized rabbit; blood-vessels of the ventral third of spleen ligatured and 6 min. of a broth culture of B. typhosus injected through the ventral end into the middle third of spleen. Autopsy after 40 hours. The ligated end of the spleen was hard, dark red and dull on the surfaces, and the hard portion was separated from the normal part by a sharp ridge. Spleen plates; I, innumerable colonies; II, 1900 colonies; III, 1150 colonies. Plates from liver developed 91 colonies; from hepatic vein, 90 colonies; from left ventricle, 20 colonies; and from left kidney, 4 colonies, of B. typhosus.

In some experiments, in which the vessels of the middle portion of the spleen were ligated and the animal was allowed to live for seven or eight days, the entire middle portion of the spleen had become very much thickened, of the greyish yellow color and remarkably dry, while both ends of the organ, the circulation in which we endeavored not to interfere with, appeared normal in every respect.

Thus we see that our expectation of a collateral interstitial nutrition was not realized, an interesting fact which, however, we cannot discuss further here.

On the other hand, our experiments show that plates from the non-ligated portions of these partly ligatured spleens developed a considerable number of colon or typhoid colonies, quite in contrast to our experience with spleens no part of which were ligated.

In studying over our entire material it seemed to us that the as-

sumptions which give the fittest interpretation of all the facts in our possession—at least for the present—are: (1) That the blood within the spleen is less bactericidal than the blood within the balance of the circulation, on account, perhaps, of the nourishment afforded the bacteria by the large numbers of disintegrated erythrocytes within the spleen; and (2) That the structure of the spleen offers but little hindrance to the rapid migration into the circulation of large numbers of bacteria injected into this organ.

Our experiments, which showed that but few bacteria could be found in the spleen only a few hours after large numbers had been injected into it, must be explained by assuming that great numbers of them were soon carried away from this organ through the splenic veins by the blood stream; the fate they met was probably that which awaits those large numbers of bacteria which are injected directly into the circulation.

In our second set of experiments, in which the bacteria were injected into a spleen, part of the vessels of which had been ligated, the presence of bacteria in the non-ligated (normal?) portion of the spleen may be explained by assuming that many of those which got into the ligated part remained there and, from the abundance of nutriment, multiplied rapidly. From this source a fresh supply of bacteria was constantly given out to the adjacent, more normal part of the spleen, whence the blood, flowing through the organ, carries the bacteria out almost as soon as they are supplied to it.

Although it seems to be a fact that but little interchange of nutritive fluids takes place between the several regions of the spleen, the bacteria are apparently able to wander over by their own motility (colon and typhoid), or to grow through, from one region into another.

The fact that, only a few hours after injection into the spleen, so few bacteria could be found in the blood, does not argue against the assumption that all the injected bacteria were carried out into the blood stream, because they disappear in the same manner when injected directly into the veins.

Wyssokowitsch * found five hours after injecting 1 cc. of typhoid

* *Ztschr. f. Hygiene*, i (1886), 1.

culture into the jugular vein of a rabbit that 0.2 cc. of the blood contained only 5 bacilli.

The presence of 1900 colonies in plates made from a point in the spleen adjacent to a part the vessels of which had been tied, while not more than 90 colonies were found in plates made from the liver and the hepatic vein, may well be interpreted in favor of the assumption that blood within the spleen is less bactericidal than blood in the vessels nearest to this organ.

Another point in favor of this assumption seems to be the fact that the bacteria thrive in a ligated spleen, while, according to de Giaxa and Guarnieri,* bacteria succumb to the bactericidal effects of blood in the vessels between two ligatures as well as in the extra-vascular blood.

Finally, we have to report that in a number of cover-glass smears made from non-ligated spleens, which were most carefully examined microscopically, no bacilli were discovered; and it would seem to us improbable, even if only a considerable portion of the bacteria were killed by the alexines, that no bodies of the bacteria, which would take a stain, should be found in the smears.

The views here put forward would seem not to be in harmony with the statements of Wyssokowitsch.† This author found, after injecting numerous micro-organisms into the jugular vein, that they soon disappeared from the circulation, but that a number of them settled in the spleen, liver and bone-marrow, a fate which, according to Ponfick ‡ and others, they share with non-organized corpuscular bodies, such as cinnabar, ultramarine, etc., injected into the circulation.

Wyssokowitsch, however, states further that certain bacteria, for example the typhoid bacillus and others, soon become destroyed within the spleen. He states that twenty-four hours after injection of the typhoid bacillus into the blood, cultures made from the spleen show no living bacilli; in other words, he claims that the spleen acts as a filter upon the organisms in the blood passing through it, and the bacilli succumb to the bactericidal influences within the spleen.

With regard to the first point we freely admit that corpuscular

* *Annales de Micrographie*, iii (1890-91), 481. † Loc. cit.
‡ Virchow's *Archiv*, 1869.

elements may find within the spleen a greater obstacle to their free movement than in any other part of the blood current, and that a larger accumulation of organisms may occur in the spleen than in any other part of the circulation; yet the results of our experiments have prompted us to assume that the retained bacteria are easily carried out again from the spleen, and that the " filtration " is comparatively of but little account and not permanent. As to the disappearance of the bacteria from the spleen, we maintain that Wyssokowitsch has not offered the slightest proof of his assumption that the destruction of the bacteria occurs within the spleen. This disappearance of the bacteria can be as well explained by supposing that they have been gradually carried out from the spleen by the blood into the vessels, where they have succumbed to the bactericidal power of the circulating blood. We wish it understood that we by no means claim that the spleen possesses no degree of bactericidal influence and that no destruction of bacteria takes place in this organ. On the contrary, at the beginning of our experiments, the total sterility of the inoculated spleens led us to believe that the bacteria were destroyed by the bactericidal elements of this organ, and when during our investigations the highly important publications of R. Pfeiffer * and of A. Wassermann † appeared, showing that the elements of active immunization in cholera and in typhoid fever appear in the greatest amounts in the spleen, lymphatic glands and bone-marrow; and inasmuch as immunization in typhoid fever and in cholera does not mean antitoxic but bactericidal effects, we were further strengthened in our belief that we, too, had found bactericidal effects of remarkable power within the spleen. Yet a careful analysis of the results of all our experiments led us to the present conclusion that the bactericidal power within the spleen acts rather less intensely than in the rest of the circulation.

As a matter of fact, this view seems to us to harmonize far better with the principles and findings of Ehrlich, Pfeiffer and Wassermann. The normal bactericidal elements are not identical with the bacteri-

* Pfeiffer and Marx, *Deutsch. med. Wochenschr.*, 1898, p. 47.
† *Berliner klin. Wochenschr.*, 1898, p. 209.

cidal bodies of active immunization; they are derived from different sources. The bactericidal elements of the normal blood are seemingly generated in one way or another by the leucocytes, while Pfeiffer has established the fact that the bactericidal bodies generated by immunization stand in no relation to the leucocytes. Following the theory of the so-called "side-chain-immunity" of Ehrlich,* it is assumed by Behring † and by Wassermann that these bactericidal bodies are generated by the organ-cells, which, reacting to certain stimuli, produce an excess of these bodies. The stimulating agents are the bacteria and their products.

This, in a few words, is the essence of the views of these writers so far as concerns us here. We believe now that we are not going too far when we add the hypothesis that the greatest overproduction of the bactericidal bodies occurs in these organs, in which the bacteria are permitted to multiply most actively. In other words, when the bactericidal elements of the normal blood are less active, less influential, then the most active generation of bactericidal bodies of immunization takes place. It is in this sense, then, we can claim that our assumption of the diminished effect of the normal bactericidal action within the spleen harmonizes with the statements of Pfeiffer and of Wassermann, that the bactericidal bodies of immunization are abundantly produced within the spleen.

In one of our experiments with injections into the non-ligated spleen, numerous bacilli were found to be present in the liver.

In this case an attempt had been made to reach the vagus nerve below the diaphragm, requiring some pressure upon the liver, and it occurred to us that the mechanical injury the liver had received might have been the cause of the retention and perhaps multiplication of the bacteria within it. We therefore attempted to produce by cauterization circumscribed lesions of the surface of the liver before injecting bacteria into the spleen, and our experiments in this line show conclusively that by mechanical injury to the tissue we have it

* Die Wertbemessung des Diphtherieheilserums, *Klinisches Jahrbuch*, vi (1897).

† *Deutsch. med. Wochenschr.*, 1898, p. 68.

in our power to cause bacteria which are circulating in the blood to settle in any part of the surface of the liver we may select. In all the cases experimented upon the cauterized part of the liver contained innumerable organisms; in the normal part of the organ immediately adjoining the lesion they were still numerous, while in remote parts but few, if any, were to be found. The organisms found in the lesions were pure cultures of the species which had been injected into the spleen. In cases in which the animal was allowed to live for 30 to 40 hours, it was easy to establish the fact that an innumerable quantity of bacteria were present in the lesion, while none were found at the site of inoculation. The perfect sterility of the spleen and the immense number of bacteria found at the site of the lesion showed that at least some of the bacteria migrated from the spleen in a living condition.

The liver, however, is the next station at which bacteria escaping from the spleen would arrive, and the question suggested itself whether a sufficient number of bacteria escaped from the spleen to allow of their reaching and settling in a more remote organ, and whether or not lesions of other organs would react in a similar manner to those of the liver.

To this end lesions were produced by cauterization in several organs: in the kidney, the uterus, the testicle, and in a circumscribed area in the peritoneum, and in the subcutaneous tissue, etc. In all places where suitable lesions were produced, the specific organisms were present in large numbers, as illustrated by the following experiments.

Experiment 12. Large rabbit; cauterized pregnant uterus through incision in abdomen and injected 2.5 min. of a 3 days broth culture of B. coli comm. into spleen. Autopsy after 3½ days. Spleen plates; I, 1 colony; II and III, 0. Plates from liver, 0; from hepatic vein, 1 colony; from left ventricle, 1 colony; from left kidney, 0; from the site of lesion in the uterus and from the fluid in the cavity of the uterus at site of lesion, innumerable colonies.

In this experiment the quantity of culture injected was small, the rabbit and the spleen were both large, and the plates made from the blood and uninjured organs were sterile—conditions which would

favor the interpretation that the bacteria were all destroyed in the spleen, but the abundant growth in the lesion of the uterus demonstrates that a considerable number of bacilli must have escaped into the general circulation to found this thriving colony in a remote region.

Experiment 13. Medium-sized rabbit; cauterized right kidney and uterus, and injected 5 min. of a three days broth culture of Staph. pyogenes aureus into the spleen. The tip of the organ through which the needle was inserted was tied. Autopsy after 40 hours. Spleen plates from tied tip showed 460 colonies; from part I, 192 colonies; part II, 7 colonies; part III, 4 colonies; plates from the liver, hepatic vein, left ventricle and left kidney were sterile; from the right kidney, necrotic portion and adjoining portion, innumerable colonies; and from the necrotic portion of the uterus over 10,000 colonies developed. The tip of the spleen contained a considerable number of bacilli, as did also the adjoining part, a fact which was observed in nearly all experiments in which the tip of the organ was tied, and which probably is explained by the cutting off of the blood supply by the ligature.

Our observations on the action of bacteria in lesions made in the internal organs seemed to be of such importance, that we conducted a series of experiments by injecting bacteria into the general circulation, after producing lesions in one or more of the internal organs.

Experiment 14. Large rabbit; ventral end of spleen and border of liver squeezed with hot forceps; 25 min. of a 20-hour broth culture of B. coli comm. injected into ear vein. Autopsy after 40 hours. The cauterized portions of the spleen and of the liver were necrotic. Plates from the blood in left ventricle showed 1 colony; from left kidney, 0; from hepatic vein, 48 colonies; from normal part of liver, 2 colonies; from the necrotic part of the liver the colonies were innumerable; from part I, of spleen, the necrotic portion, innumerable colonies; from part II the colonies were also innumerable, but distinctly fewer than were found in part I, and from part III only 39 colonies developed.

Experiment 15. Large rabbit; cauterized right kidney, border of liver, two areas in spleen, one near each end, a circumscribed area of the peritoneum, and the subcutaneous tissue in the right inguinal region. Twenty-five minims of a broth culture of B. coli communis was then introduced into the ear vein and the animal killed after 64 hours.

Plates made from blood in the left ventricle showed no growth; from hepatic vein, 0; from a normal portion of the liver, 0; from necrotic part of the liver, innumerable colonies; spleen plates showed an innumerable number of colonies from the necrotic portions and a very abundant growth, though distinctly less, from the intermediate parts; plates from the left kidney showed no growth, while those from the normal part of the right kidney showed 410 colonies; the development from the necrotic portion of the right kidney and the apparently healthy tissue adjoining it was innumerable, though distinctly less in the latter case than in the former. Plates made from the cauterized portion of the peritoneum and from the necrotic subcutaneous tissue, all showed an innumerable growth.

Experiment 16. Medium-sized rabbit; squeezed with hot forceps the ventral end of the spleen, the lower border of the liver and the lower end of the right kidney. Twelve minims of broth culture of B. coli comm. were then injected into the ear vein. The autopsy was made after 41 hours and plates from blood in the left ventricle developed no colonies; from hepatic vein, 72 colonies; from normal part of liver, 570 colonies; from the necrotic part of this organ, innumerable colonies; from an adjoining portion, 2671 colonies; from the left or normal kidney, 0; from the squeezed portion of the right kidney, innumerable colonies. Plates made from various parts of the spleen developed very few colonies.

The few colonies found in plates from the spleen may perhaps be explained by the fact that the squeezed portion of the spleen was dry and leathery in appearance and very tough when cut into, and it may be that the circulation was so completely cut off from this part that the bacteria had but little chance to reach the lesion in the organ, and were mostly carried out again into the circulation. It seems probable that only when some more or less normal tissue remains within the necrotic part, *i. e.* tissue through which the blood can flow, that the bacteria can gain access to the injured tissue and be deposited there. It is of course a very difficult matter always to regulate the amount of pressure exerted, so as to produce the same kind of a lesion in every experiment.

In this connection it seems important to mention that in experiments in which half of the vessels of the spleen were tied and the inoculation made into the circulation through the ear vein, plates made from the spleen, whether from the ligated or the non-ligated

part, remained sterile. The sterility of the ligated portion probably finds its explanation in the reasons set forth above. Injections made directly into or through a ligated portion of the spleen offer entirely different conditions, for here the bacteria are brought directly into the ischæmic part and could not escape except through their own motility or by growing out through enormous multiplication. It seems likely that the abundant growth within the ligatured portion of the spleen was due largely to the lesion of the tissue caused by ligation of the vessels which favored the retention and development of the organisms.

The principle underlying the fact that a lesion in an organ favors the retention and development of bacteria within it is not new. J. Rosenbach,* Becker,† and others, have shown that the intravenous injection of Staph. pyogenes aureus can cause osteomyelitis in a fractured bone. The experiments of Wyssokowitsch,‡ and of Prudden,§ have established that a mechanical lesion, and according to Prudden that also a chemical lesion, of the cardiac valves causes the staphylococcus and the streptococcus, when intravenously injected, to settle there and develop ulcerative endocarditis. According to Wyssokowitsch, however, the M. tetragenus and other organisms did not settle in the injured valve. In our experiments with the three species of bacteria mentioned, we made no attempt to determine how they would act in relation to injured heart valves, but were enabled to cause them to settle in any tissue experimented with, by producing a suitable lesion. This seems to us a point of great practical importance, inasmuch as any physiological or artificial injury, such as parturition or operation, carried out under the most scrupulous asepsis, can become infected, if in any place in the body there exists a focus containing micro-organisms; and in this connection it must not be forgotten that attenuated micro-organisms may regain their virulence in a tissue lesion.|| How many cases of puerperal infection or of post-operative

* *Zeitschr. f. Chirurgie*, x.
† *Deutsche med. Wochenschr.*, 1884.
‡ Virchow's *Archiv*, ciii.
§ *Amer. Journ. Med. Sciences*, 1887, p. 70.
|| Nocard and Roux, *Annales de l'Institut Pasteur*, 1887.

sepsis might here find their explanation! Without, however, entering into a discussion of the numerous instances in pathology which can be interpreted profitably by our facts, we would add that, judging from a few experiments we have made, it would appear that lesions of the internal organs can become infected also from a subcutaneous focus.

In one experiment in which subcutaneous injection of the B. coli comm. was employed, plates made from the injured portion of the liver contained over 2000 colonies, while the necrotic spleen showed a very meagre growth. In another similar experiment the plates from the necrotic portion of the spleen contained over 6000 colonies, from a cauterized portion of the peritoneum about 1000 colonies, and in plates from the cauterized uterus there were innumerable colonies.

The failure of Wyssokowitsch to produce ulcerative endocarditis from subcutaneous injections of pyogenic bacteria may perhaps be explained by the fact that certain bacteria are but slowly absorbed from the subcutaneous tissue; if the injury to the valves had been caused a few days after the injection instead of simultaneously, more time would have been allowed for the bacteria to get into the circulation, and the result might have been different.

The frequent mention of the presence of B. coli comm. in the cauterized portions of the liver might lead to the suspicion that this ubiquitous organism was present in the liver before the intrasplenic or intravenous injections were made. Aside from the numerous instances in which the uninjured organs were found free from this bacillus, other studies bearing directly on this point were made. A number of rabbits were opened, the liver and spleen were cauterized and the abdomen closed, just as in the other operations alluded to, but without any injection of micro-organisms whatever. After a few days the animals were killed, and plates made immediately from the injured and uninjured organs and from the blood, and in all the cases the plates were found to be sterile. It therefore seems to us probable that the cases in which numerous colon bacilli were found in the normal liver were from platings made some time after death had occurred.

In conclusion we desire to say that, although in our many experiments we have produced numerous foci containing very many bacteria, and thus have offered a number of different sources for the abundant supply of bacteria to the blood, the blood, nevertheless, has remained perfectly sterile or contained but very few bacteria. The blood is a liquid tissue, and may we not, perhaps, assume that the blood, like other tissues, permits the growth of bacteria within it only when it has been in some way injured?

SUMMARY.

I. Cultures of B. coli communis, B. typhosus and Staph. pyogenes aureus, when injected into the tissue of the normal spleen, soon disappear from that organ, and indeed from the normal body generally.

II. Bacteria injected into a spleen after the whole or a part of the vessels have been tied, multiply in the spleen with great rapidity and continue to supply bacteria to the blood, whence in the healthy body they soon disappear.

III. Bacteria injected into the spleen, or subcutaneous tissue, or into the blood current through the ear vein, in cases in which moderate lesions have been made by cauterization or compression in the spleen, liver, kidney, uterus, testicle, peritoneum, or subcutaneous tissue, usually find lodgment in these lesions and multiply there.

IV. Even in cases in which numerous foci existed, from which the blood was constantly provided with a fresh supply of bacteria, only few bacteria were found at any time in the blood.